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STUDIES ON
Prostaglandin

Occurence,
Formation and Biological Actions

BY

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FROM THE DEPARTMENT OF PHYSIOLOGY, KAROLINSKA INSTITUTET, STOCKHOLM

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KUNGL. BOKTRYCKERIET P.A. NORSTEDT & SÖNER

TO MY WIFE AND OUR CHILDREN

I.

II.

III.

IV.

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Introduction

ACTION OF SEMINAL FLUID ON SMOOTH MUSCLE

Human seminal fluid is known to be active on the smooth muscle of various origin. KURZROK and LIEB (1930) and COCKRILL, MILLER and KURZROK (1935) studied the effect of human seminal fluid on isolated strips of the non-pregnant human uterus. The seminal fluid usually caused a marked decrease in tonus, but in some cases an increase was observed. Since it was possible to reproduce most of the results with acetylcholine and also to inhibit the effects with atropine, it was assumed that the active principle was acetylcholine.

GOLDBLATT (1933, 1935) found that intravenous injection of human seminal fluid gave a pronounced decrease in blood pressure in rabbits and cats. He also observed that both native and alcohol extracted human seminal fluid possessed a marked oxytocic effect on the isolated guinea-pig uterus.

EULER (1934) independently found that seminal fluid of man contained a blood pressure lowering principle, which also stimulated smooth muscle organs like the isolated rabbit jejunum. He further studied the nature of the active principle of human seminal fluid and also observed that a similar principle could be extracted from various male accessory genital glands. With more purified extracts he demonstrated that the active principle could be clearly differentiated from other known smooth muscle stimulating substances on the basis of its chemical characteristics. The active principle was found to behave as a lipid soluble organic acid and was named prostaglandin (EULER 1935 b, 1936).

The effect of seminal fluid on the nonpregnant human uterus in vivo was studied by KARLSON (1949). The motility was recorded with three separate pressure pickups (KARLSON 1944) which simultaneously registered the pressure changes in the corpus, isthmus, and cervix uteri. At the time of ovulation seminal fluid produced an increased activity in the corpus, but a reduction in motility and tonus in the isthmus and the cervix. If the same experiments were performed during the proliferatory phase, an increased motility and tone in all parts of the uterus was observed.

The only smooth muscle stimulating substance that has been demonstrated besides prostaglandin in human seminal plasma is histamine.

VANDELLI (1943) investigated 16 samples of seminal plasma with Code's method (CODE 1937) and found a mean value of 2.04 μg histamine per ml (range 0 to 15 μg). There is no chemical evidence for the occurrence of acetylcholine (MANN 1954, page 173) or 5-hydroxytryptamine (ELIASSON, unpublished) in human semen.

PROSTAGLANDIN

According to EULER (1935 b, 1939) prostaglandin is *defined* as the lipid soluble smooth muscle stimulating and blood pressure lowering factor with acidic properties in seminal fluid and in extracts of some accessory genital glands of man and sheep.

The extracts used by GOLDBLATT (1935) were prepared by adding four volumes of alcohol to the seminal fluid. After centrifugation the supernatant was evaporated and the residue dissolved in water. A more pure preparation was obtained by EULER (1936, 1939) by precipitating inert material with acid alcohol or acetone and then extracting the active principle with ether from the acidified water solution. The active material could then be extracted from ether with water if the pH was adjusted to 6.8 or above. By adding barium hydroxide much inert material was precipitated and after drying a stable barium prostaglandin preparation was obtained. Further purification of this Ba-salt preparation was obtained by BERGSTRÖM (1949) who used chromatography and countercurrent distribution, and obtained an extract, whose biological activity per weight was comparable to that of acetylcholine on the isolated rabbit jejunum. The methods used have not been published in detail.

Prostaglandin has also been extracted from human seminal fluid and from the sheep's vesicular glands with hot acidified alcohol (ELIASSON 1957). After concentration under reduced pressure and after adding sodium hydroxide the unsaponified material was extracted with ether, leaving the prostaglandin in the water solution. The active principle was then extracted with ether at an acid pH and separated from the saturated fatty acids by the lead acetate method (HILDITCH 1949, p. 468).

According to EULER (1934) prostaglandin could be extracted from seminal fluid of man and sheep, from the prostate gland and seminal vesicles of man, and from the vesicular glands of sheep. On the other hand it was not demonstrated in the analogous secretions or organs of other investigated animals, like bull, boar, dog, rabbit, etc. Of the several organs of sheep investigated the active principle was only obtained in significant amount from the vesicular glands (EULER and HAMMARSTRÖM 1937).

In 1939 EULER defined his *unit of prostaglandin* as the activity of 0.1 mg

of a barium salt preparation of prostaglandin. Intravenous injection of one unit in rabbits, pretreated with atropine, usually produced a decrease in the blood pressure of about 30 per cent.

Using this definition he found that the *amounts of prostaglandin* in sheep's vesicular glands corresponded to about 2—5 units per gram tissue (Euler 1939). In seminal fluid of normal young men the prostaglandin activity often corresponded to 20—30 units per ml (Euler 1949). Samples obtained from patients attending the sterility clinics were found to be less active, 8.3 units being the mean value for 155 samples (Asplund 1947 a).

As to its *chemical properties* prostaglandin was shown to be a lipid soluble substance (Euler 1935 b, 1936). Further investigations showed that prostaglandin is a nitrogen-free carboxylic acid, probably unsaturated and with hydroxy groups (Euler 1936, 1939, Bergström 1949). The free acid is only slightly soluble in water and petroleum ether, but freely soluble in organic solvents like ethanol, acetone, ether and ethyl acetate. It forms water soluble compounds with sodium, potassium and calcium and also with some metal ions like barium, cadmium, copper, mercury and zinc. It is dialysable through cellophane, parchment and collodion and in an electric field at approximately neutral pH prostaglandin moves towards the anode. Prostaglandin has been reported to resist boiling for 20 minutes at pH between 1 and 7, the activity being rapidly decreased on both sides of this range (Euler 1936, 1939). Eliasson (1957), using a different extraction method, found his prostaglandin preparation to be resistant towards boiling for 20 minutes in the pH range 3 to 9.

The absorption spectrum in the ultraviolet region of highly purified preparations of prostaglandin shows a marked absorption band at 2,800 Å (Euler 1936, Bergström 1949).

The *biological properties* of prostaglandin purified from human seminal fluid and from sheep's vesicular glands were extensively studied by Euler (1936, 1939). Smooth muscle organs like the intestine (rabbit, guinea-pig, rat, mouse, squirrel) and the uterus (cow, rabbit, guinea-pig, rat) were found to be stimulated. When given intravenously to anaesthetized rabbits, cats or dogs it produces a protracted fall in blood pressure. Prostaglandin causes a pronounced vasoconstriction in perfused lungs, liver and placenta, but has only a slight effect on other vascular beds. In the nonanaesthetized rabbit prostaglandin also produces a fall in the blood pressure as measured in the T-tube cannulated common carotid artery (Eliasson, unpublished observation).

According to Asplund (1947 b) prostaglandin and native human seminal fluid administered in the vagina or injected intravenously produce an increased activity of the rabbits uterus, but a decrease in the tonus of the abdominal tubal ostium.

Prostaglandin extracted from human seminal fluid and from vesicular glands of sheep was found to have quantitatively the same effects on rabbit blood pressure, and on the isolated intestine and uterus of the rabbit. The two preparations were also found to be inactivated in the same way by acids and alkali (EULER 1936). This was confirmed by ELIASSON (1957) who found that prostaglandin extracted from these two sources also were similar with regard to the effects on isolated intestine and uterus of the rat and guinea-pig. On paper chromatography with different solvent combinations they behaved identically.

The smooth muscle stimulating action of prostaglandin on rabbit jejunum is not influenced by atropine in doses which completely block the effect of acetylcholine (EULER 1936, 1939, ELIASSON 1957). On the guinea-pig ileum large doses of atropine diminish the prostaglandin response (VOGT 1958 b, ELIASSON 1959), but the doses of atropine required are higher than those needed to block the effect of acetylcholine (ELIASSON 1959). The stimulating effect of prostaglandin is not inhibited by specific doses of antihistaminics, hexamethonium, d-tubocurarine, nicotine, cocaine, tryptamine, lysergic acid diethylamide (LSD-25) or dihydroergotamine. In some of the experiments it was found that hexamethonium and cocaine, in doses which completely blocked the effect of stimulating doses of nicotine, enhanced the response to prostaglandin (ELIASSON 1959). The only substance found, which inhibited the stimulating action of prostaglandin was patulin, which also inhibited the effects of acetylcholine, histamine, 5-hydroxytryptamine and nicotine, The same doses of patulin did not alter the response to barium chloride. and in most cases enhanced the effect of substance P (ELIASSON 1958 b).

On account of his investigations EULER (1936) suggested, that the *physiological function* of prostaglandin might be that of an autonomic regulator for emptying of the accessory genital glands, *i. e.* when the smooth muscle stimulating principle has accumulated sufficiently it would act as a stimulus for the emptying of the glands.

In the 155 specimens of semen from infertile patients examined by ASPLUND (1947 a) more than 40 per cent contained less than 5 units prostaglandin per ml. This observation seems to be in accordance with the results by HAWKINS and LABRUM (1956, 1959), who found a correlation between the concentration of prostaglandin in patient's seminal fluid and the ease with which conception is achieved.

When the concentration of prostaglandin in various specimens of seminal fluid was related to other properties of the samples HAWKINS and LABRUM (1956) found a correlation to the maintenance of the spermatozoal activity. Such a correlation was, however, not obtained by ASPLUND (1947 a). In none of the two investigations there was found any correlation between the prostaglandin concentration and the volume,

sperm concentration, percentage motile spermatozoa, or the degree of activity of the spermatozoa in the specimens.

The results obtained from the various investigations on prostaglandin strongly suggest that this principle plays a role in reproduction physiology in man. The physiological function is, however, at present not known.

Two smooth muscle stimulating compounds with acidic properties have recently been crystallized from the vesicular glands of sheep (BERGSTRÖM and SJÖVALL 1957, 1959). The two compounds have been named prostaglandin E and F (PGE and PGF). Chemically they have been found to differ from the partially purified prostaglandin preparations by having no absorption at 2,800 Å (BERGSTRÖM, personal communication).

Some of the biological effects of these two preparations have been studied on several isolated smooth muscle organs as well as the blood pressure in man and rabbit (BERGSTRÖM *et al.* 1959 a, b). On various organs PGE and PGF showed different activity ratio. PGE produced a definite lowering of the rabbit's blood pressure when injected in doses of 0.5 μg, while 10 μg PGF was without any effect. On guinea-pig ileum and chicken jejunum PGE was also more potent than PGF, while the opposite was the case on rabbit's and rat's uteri. Prostaglandin was compared with PGE and PGF on the same test organ, and from the results it was concluded that prostaglandin is identical neither with PGE nor with PGF. The possibility that prostaglandin might be a mixture of PGE and PGF was also discussed, but from the data obtained it seemed unlikely that the biological activity of prostaglandin was due to a mixture of PGE and PGF. The true nature of prostaglandin is still unknown.

In the present investigation EULER's definition of prostaglandin (1935 b) is used, *i. e.* the acid, lipid soluble, smooth muscle stimulating and blood pressure lowering principle present in seminal fluid and extracts from some of the accessory genital glands of man and sheep. The occurrence, origin, formation and biological characteristics of this principle have been studied.

General Methods

PREPARATION OF PROSTAGLANDIN

Extraction of human seminal fluid. Samples were obtained from the sterility clinics at the Karolinska and Sabbatsberg's Hospitals. The surplus from the routine analyses of the delivered ejaculate was deep frozen (usually within four hours after ejaculation).

Each sample of seminal fluid, mixed with 2 volumes of acidified acetone (1 ml 3 N HCl per 100 ml) was heated to boiling, filtered and left in a refrigerator (0° C) overnight. After refiltration, the filtrate was evaporated under reduced pressure to a volume corresponding to $^1/_3$ or $^1/_4$ of the original volume of the seminal fluid.

This volume was thoroughly extracted twice with $^1/_2$ volume of acidified ethyl acetate (1 ml 3 N HCl per 100 ml) and a third time with $^1/_2$ volume of ethyl acetate saturated with water. The extraction was made by mechanically shaking the solution for 15 minutes at a pH of less than 3. The combined ethyl acetate portions were then extracted with three portions ($^1/_2 + {}^1/_3 + {}^1/_3$ volume) of 0.15 M phosphate buffer (pH 7.5) at a pH between 7 and 8. The pH was adjusted with 5 N NaOH.

When a more concentrated prostaglandin solution was desired the combined ethyl acetate portions were partially evaporated under reduced pressure before extraction with phosphate buffer.

The buffer extracts were combined and the ethyl acetate evaporated off. When it had been necessary to concentrate the phosphate buffer extract further to obtain the desired concentration, the solution was then left in a refrigerator overnight and the precipitated salt discarded by centrifugation and pH adjusted to about 7.5.

Extraction of organs. The organs were removed shortly following death and deep frozen by dry ice and stored at — 20° C. For the routine work with prostaglandin only the sheep's vesicular glands were used. In this case there was usually a time delay of 30—60 minutes between the death of the animal and storage of the organ in the deep freezer (— 20° C).

The cleaned organs were minced in a mincing machine and mixed with two volumes of acidified acetone (1 ml 3 N HCl per 100 ml). The thermostable mixture was heated to boiling, after filtration the solution

was put in a refrigerator overnight, refiltered and then evaporated under reduced pressure to a volume corresponding to about 2—10 gram organ per ml. The prostaglandin activity was then extracted with ethyl acetate and 0.15 M phosphate buffer as described above.

Control extraction from ground vesicular glands to which 25 to 100 units prostaglandin had been added, gave recovery of 60 to 80 per cent.

The bioassay of duplicate extractions of various samples gave results which agreed to within 5 to 10 per cent.

PROSTAGLANDIN STANDARD

In his experiments Euler (1936, 1939) used a stable Ba-salt of prostaglandin as a standard, 0.1 mg corresponding to one unit. As there was no prostaglandin standard available at the beginning of this work, a new one was prepared according to the following method (Eliasson 1957). The cleaned and ground vesicular glands of sheep were mixed with two volumes of acidified ethanol (1 ml 3 N HCl per 200 ml 95 % ethanol). After heating to boiling the solution was filtered and the filtrate put in a refrigerator over night, refiltered, then evaporated under reduced pressure until 1 ml filtrate contained the equivalent of 20—30 gram tissue. During slight warming of the solution the pH was adjusted to about 7.5 with sodium hydroxide. The unsaponified matter was extracted three times with 10 volumes of ether. These ether fractions did not contain any activity and were discarded. 6 N HCl was added to pH 3 and the water phase again extracted three times with 10 volumes of ether. The ether was separated from the water phase and evaporated under reduced pressure. The residue was dissolved in a small volume of 0.1 N NaOH, pH adjusted to about 7 and 0.15 M phosphate buffer at pH 7.5 added to make up the desired volume.

The solution was poured into ampoules which were sealed in vacuo and then stored at — 20° C.

Stored three years old preparations showed no inactivation.

Although the extraction method used excludes all basic substances like acetylcholine, histamine, 5-hydroxytryptamine, and substance P, the specificity of the standard with regard to the occurrence of biologically active substances other than prostaglandin was tested after subjecting the solution to paper chromatography. This was performed on Whatman no. 1 paper and the solvents used were: methylethylketone/diethylamine/water (60 : 3 : 20); ethylacetate/acetic acid/water (3 : 1 : 1); and methylglycol/conc. NH_4OH/water (80 : 5 : 15). The paper was dried, cut into 3 × 1 cm strips and each strip immersed in an intestinal bath using the

isolated rabbit jejunum as test organ. In no case was there more than one peak of activity found (ELIASSON 1957).

DEFINITION OF THE PROSTAGLANDIN UNIT USED IN THE PRESENT INVESTIGATION

Because of lack of Ba-salt standard, it was decided that the amount of the new standard solution which gave a blood pressure fall of approximately 30 per cent, when injected intravenously in urethane anaesthetized rabbits (1.5 g/kg) pretreated with 2 mg atropine and 1 mg phenbenzamine (Lergitin®, Recip) per kg, corresponded to 1 unit.

In a few experiments it was later possible to compare the effects of the present prostaglandin preparation with the Ba-salt preparation previously referred to. On the rabbit's isolated jejunum 1 unit prostaglandin corresponded to 0.15 mg of the Ba-salt of Euler's prostaglandin standard (i. e. 1.5 unit) (EULER 1939).

BIOLOGICAL ASSAY

Rabbit's blood pressure. The animals were anaesthetized with urethane (1.5 g/kg) intravenously. Atropine (2 mg/kg) and phenbenzamine (Lergitin®, Recip; 1 mg/kg) were given subcutaneously. The blood pressure was recorded from the common carotic artery with a mercury manometer. The test samples were given into the femoral or external jugular vein in doses between 0.05 and 0.5 ml followed by 1 ml Ringer solution.

Rabbit's intestine. The animals were anaesthetized with urethane, and after 15—20 minutes bled to death. About 15 cm of the duodenum-jejunum was removed and placed in Tyrode solution. A strip of about 2—3 cm was suspended in a 15 ml bath containing Tyrode solution aerated with 6.5 per cent carbon dioxide in oxygen and kept at 38° C.

Guinea-pig's ileum. The animals were killed by a blow on the head and about 15 cm of the distal part of the ileum was removed and placed in modified Tyrode solution. A segment of about 2—2.5 cm length was fixed in a 3 ml organ bath, the solution aerated with oxygen and kept at 38° C. This Tyrode solution had the following composition: 0.8 per cent NaCl; 0.02 per cent KCl; 0.02 per cent $CaCl_2$; 0.01 per cent $MgCl_2 \cdot 6H_2O$; 0.1 per cent $NaHCO_3$; 0.005 per cent $NaH_2PO_4 \cdot H_2O$; and 0.1 per cent glucose.

Rat's jejunum. The animals were killed by a blow on the head and about 10 cm of the jejunum removed and placed in Tyrode solution. A strip of about 3—4 cm was suspended in a 3 ml organ bath with Tyrode solution at 38° C and aerated with 6.5 per cent carbon dioxide in oxygen.

Rat's uterus. Spayed rats were given 2 μg oestradiol-17 β (Schering) in propylene glycol, three consecutive days before being used. They were killed by a blow on the head, the uterus suspended in a 3 ml organ bath with Tyrode solution aerated with 6.5 per cent carbon dioxide in oxygen and kept at 30° C. The Tyrode solution was of the same composition as for guinea-pig's ileum, but with only 0.006 per cent $CaCl_2$.

The isolated test organs were suspended in a temperature controlled organ bath and the movements recorded isotonically with a linear frontal-writing lever. Four or six point assay was performed on the isolated organs. Atropine and phenbenzamine (Lergitin®, Recip) (10^{-7} g/ml) were added when unpurified or only slightly purified extracts were tested, since it was found in control experiments that the extraction method used excluded acetylcholine and histamine.

Results

1. OCCURRENCE OF PROSTAGLANDIN

Human seminal fluid was obtained at random from 16 infertile patients. The activity was extracted and tested on atropinized isolated rabbit jejunum according to the methods described on page 6. The mean value found was 12 (range 1.5—30) prostaglandin units per ml, while tests on some of the unpurified samples generally showed 10—20 per cent higher values.

Seminal fluid obtained from two normal fertile men had a prostaglandin concentration of 40 and 50 units respectively per ml. The same high activity has also been recorded in some of the experiments described below (page 12 and 17).

Seminal fluid from sheep was collected by using an artificial vagina[1]. Two or three successive ejaculates were combined and deep frozen until used.

Owing to the small volumes of the combined samples (0.3—1.5 ml) these were diluted with three volumes of distilled water, centrifuged and tested without purification on the atropinized isolated rabbit jejunum.

The smooth muscle stimulating activity of 4 different samples from one ram, about 10 years old, corresponded to 5, 8, 12 and 20 prostaglandin units per ml respectively and the activity in 4 samples from another ram, 5 years old, corresponded to 28, 43, 55 and 55 units per ml.

Various samples of sheep seminal fluid were also combined and then extracted according to the methods used for human seminal fluid. The smooth muscle stimulating activity assayed on isolated rabbit jejunum corresponded to 20 units of prostaglandin per ml. Qualitatively the action of these prostaglandin extracts were different from the prostaglandin standard on the isolated rabbit jejunum by giving rise to a much faster initial increase in tonus (cf. page 31 and Fig. 11).

Seminal fluid from other animals (horse, oxen, goat, pig) were also obtained by using an artificial vagina[1]. Smooth muscle stimulating activity was only found in the sample from a goat in which the activity corresponded to 12 units per ml. Seminal fluid from stallion, bull and boar were inactive.

[1] The seminal fluid was kindly put to my disposal by Dr. A. Bane, Veterinary College, Stockholm.

Autopsy **human prostate gland and seminal vesicles** were extracted according to the method described on page 6. The results were, however, not reliable since these preparations also contained substances which seriously disturbed the biological assay. It did not prove possible to obtain organs without post mortem changes. Partly for this reason another method was used for investigating the origin of prostaglandin in man (page 12).

The following **accesssory genital organs from ram** were investigated: Cowper's glands, prostate glands, vesicular glands, and ampullae ductus deferens. The terminology is according to DISSELHORST (1904).

The prostate glands in sheep are localized in the posterior wall of the urethra between the Cowper's and the vesicular glands and have been regarded by some investigators as urethral glands (DISSELHORST 1904, p. 350—351). The anatomy is illustrated in Fig. 1.

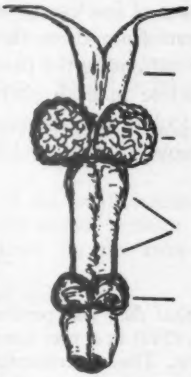

Fig. 1. Posterior view of the accessory genital glands of ram. Amp. = ampulla ductus deferens, Gl. ves. = vesicular glands, Gl. pr. = region of the urethra containing the disseminated prostate glands, Gl. Cowp. = Cowper's glands.

The organs were cleaned, ground in a mortar with quartz sand in acidified acetone, and extracted according to the method described on page 6. The extracts were tested on the isolated, atropinized rabbit jejunum and the activity per gram organ (wet weight) was found to correspond to:

Ampulla ductus deferens	2.0—2.4 units
Vesicular glands	2.4—2.7 units
Prostate glands	1.5—2.0 units
Cowper's glands	0.5 units

Male accessory genital glands from other animals, including oxen, pig, dog, cat, rabbit and guinea-pig, were also extracted, but these organs were not found to contain any prostaglandin-like activity.

2. ORIGIN OF PROSTAGLANDIN IN MAN

Because of the considerable post mortem changes in the available prostate glands and seminal vesicles from man the site of formation had to be found by an indirect method.

It is known from the work by BROESIKE (1911), GUTMAN and GUTMAN (1941), HANSEN (1946), LUNDQUIST (1949) and other investigators that during ejaculation the three main glandular systems contributing to the ejaculation are discharged successively.

The first portion of the ejaculate comes from the prostate gland and contains the main bulk of the acid phosphatase, which is secreted solely from this organ. The second portion contains the secretions from the testes, the epididymis, the vas deferens, and the ampulla and therefore contains the highest concentrations of spermatozoa. The third portion consists mainly of the secretion from the seminal vesicles and contains the highest concentration of fructose, a substance specific for this organ.

The secretions from these three glandular systems become more or less mixed. By estimating the concentration of acid phosphatase, spermatozoa and fructose in each portion of a split ejaculate it is, however, possible to calculate the amounts of the secretions from each of these glandular systems (LUNDQUIST 1949).

Methods

Collection of seminal fluid was performed according to the method described by LUNDQUIST (1949) in a tray containing six small ($5 \times 5 \times 2.5$ cm) boxes of Perspex plate. The experimental subjects were instructed to collect the ejaculate in the tray in four to six fractions of approximately the same size, i. e. the first fraction caught in box number one, the second fraction in box number two and so on. Immediately after the ejaculation the containers were closed by a close fitting lid in order to prevent evaporation.

The weight of each ejaculated fraction was determined and twice the weight or more of distilled water was added to each box to make each fraction at least 1 ml. The content was quantitatively transferred to small glass tubes, which were sealed with a rubber stopper, and placed in a deep freeze (— 20° C). The analyses were performed within a week.

Determination of acid phosphatase. A small volume of the diluted seminal fluid (0.025—0.05 ml) was further diluted (2,000—12,000 times) with distilled water according to the amount of enzyme to be expected. The analyses were done according to LUNDQUIST's (1949) modification of the method described by GUTMAN and GUTMAN (1940).

One ml of the diluted seminal fluid was incubated at 37° C in 10 ml of a citrate-HCl-buffer at pH 4.9 containing 0.005 M disodium-monophenyl phosphate. The enzymatic process was stopped after exactly 15

minutes by the addition of 1.0 ml Folin-Ciocalteu's phenol reagent. 3 ml Na_2CO_3 (20 per cent, w/v) was added and the blue colour allowed to develop in the water bath (37° C) for 30 minutes. The colour intensity was read in a spectrophotometer, model Beckman B, at 6,000 Å. The activity was expressed as units, one unit being the amount of enzyme, which under the given conditions liberates 1 mg of phenol in 1 hour.

Two standard samples (containing 0.1 mg phenol) and two blank samples (water substituted for seminal plasma) were always determined simultaneously with the analyses. The duplicate determinations agreed within $\pm$ 3 per cent.

Counting of spermatozoa. 0.05 ml of the diluted seminal fluid was mixed with 1.95 ml dilution solution (Na_2CO_3, 5 per cent; formaline, 1 per cent; and distilled water 94 per cent). A suitable amount of the thoroughly mixed solution was transferred to a Bürger counting chamber. This chamber was then left in a moist cabinet for 20—30 minutes before counting the cells.

Determination of fructose. The protein was precipitated with cadmium sulphate according to the method described by LUNDQUIST (1949). After centrifugation, 0.5 ml of the clear supernatant was transferred to a test-tube and 0.5 ml indol reagent (25 mg indol in 100 ml 0.2 per cent benzoic acid) + 5.0 ml concentrated HCl added. The test-tubes were left in a water bath at 50° C for 20 minutes, and then cooled in a flowing tap water bath. The yellow colour was read in a spectrophotometer, model Beckman B, at 4,700 Å (KARVONEN and MALM 1955).

Duplicate determinations of blank and standard samples (0.025 mg fructose) were made simultaneously. The duplicate analyses agreed within $\pm$ 3 per cent.

Determination of prostaglandin. Because of the small volumes available in some of the experiments all samples were tested, without purification as to their smooth muscle stimulating activity on the atropinized isolated rabbit jejunum.

A four point assay was performed in all cases.

Results

Seminal fluid from six normal unmarried medical students were examined. The seminal fluid from one subject (exp. IV) did not give a dose response curve, which fitted to that for the prostaglandin standard, and was therefore excluded. In this case there apparently was an inhibitory factor present in the seminal fluid. In the remaining five cases there were good agreement between the slope of the dose response curve for the different seminal samples compared with that for the standard curve.

The concentrations of the different factors investigated are summarized in Table I, and in Fig. 2 the prostaglandin concentration is plotted against that of fructose. From the results obtained it is clear that prostaglandin is liberated into the semen from the same organs as is fructose, *i. e.* the seminal vesicles.

Table I. Concentration of various constituents in different portions of human seminal fluid

Number of exp.	Number of fract.	Weight grams	Phosphatase units/ml	Spermatoz. $\times 10^6$/ml	Fructose mg/100 ml	PG units/ml
I	1	0.30	7 360	16.8	0	$<$ 1.5
	2	0.41	1 680	130	122	30
	3	0.77	730	159	185	40
	4	0.24	480	—	210	40
	5	0.37	460	54.0	240	50
II	1	0.06	14 800	0	0	0
	2	1.17	4 320	186	340	8
	3	0.61	1 400	79.2	550	10
	4	0.24	1 840	47.0	1 100	16
	5	0.54	900	9.0	760	12
III	1	0.86	2 450	97.2	63	30
	2	0.62	2 300	30.0	100	36
	3	0.45	2 110	32.4	113	45
	4	0.62	1 080	10.8	150	53
	5	0.39	670	12.6	160	53
	6	0.27	860	11.2	160	53
V	1	0.84	360	0	680	24
	2	1.08	2 450	56.4	465	22
	3	0.33	2 340	18.6	490	25
	4	0.47	1 535	5.4	610	24
VI	1	0.40	11 640	237	90	2.5
	2	0.58	4 000	80.4	200	16
	3	1.07	2 640	8.4	380	20
	4	1.20	720	0	480	25
	5	0.58	335	0	550	28
	6	0.38	510	0	510	25

The conclusion that the prostate gland does not liberate prostaglandin is specially substantiated by the results obtained in experiments I and II, in which the first fraction contains high concentration of acid phosphatase but no prostaglandin activity. This is confirmed by GOLDBLATT's observation (1935) that adenomatous human prostates did not contain any biological activity similar to that found in seminal fluid.

The concentration of acid phosphatase in the various fractions of the seminal fluid from subject V differed from the normal and indicated that he has or had a prostatitis (HANSEN 1946). Upon inquiry he admitted that 6 months ago he had been treated for an unspecific prostatitis which now was regarded as cured.

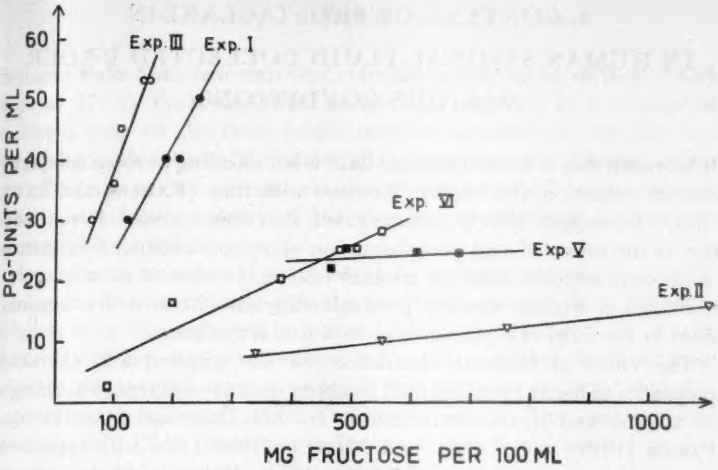

Fig. 2. Correlation between prostaglandin (PG) and fructose in various fractions of human seminal fluid. The close correlation indicates that these substances are secreted from the same organ, *i. e.* the seminal vesicles.

3. CONTENT OF PROSTAGLANDIN IN HUMAN SEMINAL FLUID COLLECTED UNDER VARIOUS CONDITIONS

It is known that if human seminal fluid is left standing at room temperature its content of free choline increases with time (KAHANE and LEVY 1937). LUNDQUIST (1947) demonstrated that this increase is partially due to the action of acid phosphatase on phosphorylcholine, a substance most likely secreted from the seminal vesicles. In view of these observations it was studied whether prostaglandin was liberated into seminal fluid in the form of a precursor or in a free active state.

The effect of frequent ejaculation on the physical and chemical properties of human seminal fluid has been studied with regard to change in volume and sperm concentration by MACLEOD and HEIM (1945), FARRIS (1949), and LAMPE and MASTERS (1956), and with regard to change in fructose content by MANN (1954). Volume and sperm concentration did not seem to be markedly different in series of specimens from normal fertile man, but a reduction in both volume and sperm concentration was often observed in successive samples from subfertile men. On the other hand it appears that a single ejaculation to a large extent depletes the seminal vesicles of its fructose content. A period of about two days is by MANN (1954) considered to be necessary for fructose in seminal fluid to reach the normal level again.

In a limited number of cases the effect of frequent intercourse on the content of prostaglandin in successive samples of human semen has therefore been studied in the present investigation.

Material and methods

Collection of seminal fluid was made from normal fertile men between 25 and 35 years old according to two different methods. *Method I:* The seminal fluid was collected in glass bottles without and with preaddition of known amounts of acidified acetone or 5 per cent trichloracetic acid. The collection of the samples was preceded by a period of abstinence for at least 5 days. *Method II:* The seminal fluid was collected by coitus condomatus, transferred to glass bottles and kept at — 5° C until the next day. The samples were then stored at — 20° C and prostaglandin extracted within a week according to the method described on page 6, with the exception that ether instead of ethyl acetate was used as organic solvent. Bioassay was performed on the isolated rabbit jejunum, atropine being added (10^{-7} g/ml) only when the native or slightly purified samples were tested.

Results

Seminal fluid from two men was collected according to method I and kept at 37° C. Each unpurified sample was subjected to a number of repeated tests on the same rabbit intestine preparation, the first test beginning within 10 to 20 minutes after the ejaculation. During a time period of up to 360 minutes no change was observed in the smooth muscle stimulating activity of the samples, as expressed in terms of prostaglandin units.

In order to elucidate if there could be a rapid increase in activity during the first ten minutes after the ejaculation, samples from the same subjects were also collected in weighed bottles containing 10 ml of acid acetone or 5 per cent trichloracetic acid. After addition of 10 volumes of ether the samples were centrifuged, the sediment discarded and the supernatant evaporated under reduced pressure. The residue was dissolved in 0.15 M phosphate buffer at pH 7 to 8 and assayed on the atropinized rabbit jejunum. The activity in both cases was of the same order of magnitude as in the control samples collected after the same abstinence period and mixed with acetone or trichloracetic acid 20—30 minutes after the ejaculation, i. e. 30 to 50 units per ml.

The effect of frequent intercourse on the concentration of prostaglandin in successive samples of human seminal fluid was studied in three different subjects. The samples were collected and extracted according to method II above. The results are summarized in Table II. The samples from the first experiments were not quantitatively transferred to glass bottles and the total amount of prostaglandin has therefore not been calculated.

The results strongly suggest that prostaglandin is liberated in an active

Table II. Concentration of prostaglandin (PG) in human seminal fluid collected at successive days. Each experimental series were preceded by a period of abstinence of 5 to 7 days

Individual	Day no.	PG units/ml	PG total	Individual	Day no.	PG units/ml	PG total
I	1	55	—	II B	1 [1]	37	150
	2	11	—		1 [1]	60	144
	5	27	—		2	45	86
II A	1	40	—	III	1	10	44
	2	17	—		2	15	45
	3	12	—		7	20	54
	7	11	—				

[1] 14 hours interval between these samples.

form and that no further formation in the ejaculated seminal fluid occurs. From Table II it is clear that in two experiments there was no significant change in the amounts of prostaglandin in the samples collected within 14 to 24 hours. The total volume of the ejaculates in experiments I and II A is not known, but several investigations show that the volume of ejaculates collected at a 24 hours interval is unchanged or lowered (MacLeod and Heim 1945, Farris 1949, Lampe and Masters 1956) and it can therefore be concluded that in these two experiments there was a significant decrease in the prostaglandin content.

4. FORMATION OF PROSTAGLANDIN IN VITRO

Using the extraction methods described above it was found that the amount of prostaglandin in human or sheep seminal fluid is much larger than in the ram's accessory genital glands. It therefore seemed pertinent to investigate if larger amount of prostaglandin could be obtained from the organs by using other extraction procedures.

It has previously been reported (Eliasson 1958 a), that mincing vesicular glands in phosphate buffer before the addition of acidified acetone enhanced the yield from about 2.5 to 15 or more prostaglandin units per gram. The amounts which could be extracted from the incubated mixture also increased with time suggesting an enzymatic process. The addition of cobra venom (Naja naja; 10 μg per ml) gave a further increment of yield of approximately 100 per cent. These preliminary experiments indicated that one could obtain prostaglandin-like activity from the sheep's vesicular glands in amounts corresponding to those found in the seminal fluid.

In this chapter the results of further studies regarding the formation of prostaglandin in vitro will be presented.

Material and methods

The organs, as described in general methods, were immediately obtained after death of the animal, deep frozen, freed of adherent fat tissue, minced in a mincing machine or Waring blendor at — 10° C and then stored at — 20° C.

The incubation medium was 0.15 M phosphate buffer at pH 7.5 unless otherwise stated. The incubation vessels were continuously shaken in a water bath at 37° C. The reaction was stopped by adding two volumes (v/v) of acidified acetone (1 ml 3 N HCl per 100 ml) and immediately immersing the reaction vessels into boiling water. As soon as the solution was boiling the vessels were removed and cooled under tap water. The solution was then filtered through a Büchner funnel by suction, the acetone evaporated under reduced pressure and pH adjusted to approximately 3 with hydrochloric acid, then extracted with ethyl acetate and phosphate buffer as described on page 6.

The samples were stored at — 20° C until bioassayed. If not otherwise stated the assay was performed on isolated rabbit jejunum. It was found that repeated freezing and thawing, or storage at temperature above freezing point decreased the activity in the samples.

Special substances used:

Cobra venom (Naja naja) was generously supplied by Dr. R. Enderlein, Lund. The venom was dried under nitrogen and stored at — 20° C.

Lecithinase A (phospholipase A) and *acetylcholinesterase*, purified by zone-electrophoresis or partition chromatography from the dried, frozen venom

of the South African ringhals cobra (Hemachatus haemachates) (Björk and Boman 1959) was kindly made available by Dr. W. Björk and Dr. H. Boman, Institute of Biochemistry, Uppsala.

The *human serum fractions IV-1 and IV-6*, prepared by the method of Cohn (Augustinsson and Heimbürger 1954) was kindly supplied by the AB Kabi, Stockholm.

Polyphloretin phosphate was prepared according to the method by Diczfalusy *et al.* (1953) and kindly supplied by the AB Leo, Hälsingborg.

Diisopropyl fluorophosphate (DFP) and *diethyl p-nitrophenyl phosphate* (E 600; Mintacol) was kindly placed at my disposal by Dr. B. Holmstedt; and *compound 48/80*, a condensation product of p-methoxyphenylmethylamine with formaldehyde, by Dr. B. Lagergren, Department of Pharmacology, Karolinska institutet, Stockholm.

All other reagents used were commercial preparations.

Results

I. Experiments with sheep's vesicular glands

Incubation in different amounts of buffer. The vesicular glands of sheep were homogenized in two volumes of buffer with a Waring blendor and incubated at + 37° C for varied periods of time. A control sample was left in a refrigerator (+ 5° C) for 180 minutes before adding acidified acetone. The increase in activity is illustrated in Fig. 3 and the result suggests an enzymatic process being involved.

The decrease in the velocity of formation of prostaglandin illustrated in Fig. 3 can theoretically be the result of many phenomena. Since the experiments with different enzymes (cf. below, Table IV) indicated that a decrease in substrate concentration probably was not the only cause, it was considered likely that increasing concentrations of formed products might exert an inhibitory action upon the reaction. In order to elucidate this point the ground vesicular glands were incubated for 60 minutes in different amounts of buffer. The results, summarized in Table III, show that the yield of prostaglandin is highly influenced by the amount of buffer. Since the alkali salt of prostaglandin is freely

Table III. Yield of prostaglandin (PG) from sheep's ground vesicular glands incubated in different volumes amounts of phosphate buffer at pH 7.5. Incubation 60 min. Temperature 37° C

Volumes of buffer (v/w)	PG-units per gram	Volumes of buffer (v/w)	PG-units per gram
2	30–40	6	80–100
4	70–95	10	60– 80

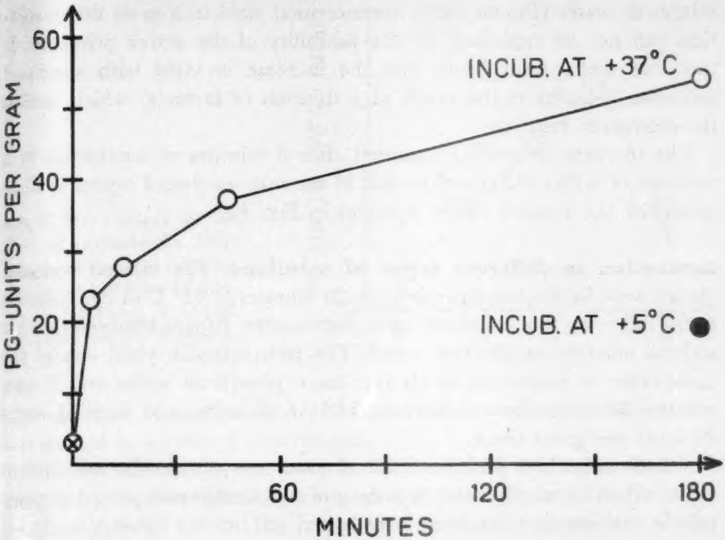

Fig. 3. Increase in yield of prostaglandin (PG) from sheep's ground vesicular glands incubated in 2 volumes of 0.15 M phosphate buffer at pH 7.5. Temperature 37° C.

⊗ = non-incubated control. ● = incubated at 5° C.

Fig. 4. Effect on the isolated rabbit jejunum of prostaglandin extracted from non-incubated and incubated ground vesicular glands of sheep. Bath volume 15 ml.

Extract from non-incubated organ: 1 = 20 mg tissue, 2 = 100 mg tissue.

Extract from organ incubated for 5 min in 5 vol. of buffer: 3 = 20 mg tissue.

soluble in water (EULER 1936) the increased yield in a more dilute solution can not be explained by the solubility of the active principle. It therefore seems most likely that the increase in yield with increased amounts of buffer is the result of a dilution of factor(s) which inhibit the enzymatic reaction.

The increase in activity obtained after 5 minutes of incubation in 5 volumes of buffer compared to that in the non-incubated organs is illustrated by the isolated rabbit jejunum in Fig. 4.

Incubation in different types of solutions. The minced vesicular glands were incubated separately for 20 minutes at 37° C in five volumes of the following four solutions: phosphate buffer, Ringer solution, isotonic sodium chloride or distilled water. The prostaglandin yield was of the same order of magnitude in all four cases: phosphate buffer and Ringer solution 30 units; isotonic sodium chloride 22 units; and distilled water 26 units per gram tissue.

Whether the high yield in distilled water was due to the salt content of the organ or whether the rupturing of cell membranes played a major role in this case has not been investigated (cf. below; Table V).

Incubation at different temperature and pH-values. Five grams of minced glands were incubated in 10 ml buffer at various temperatures for 60 minutes. The optimum temperature was 37° C ± 5° C (Fig. 5). If the same amount of organ was incubated for 60 minutes in 10 ml buffer at 37° C at various pH, it was found that maximum yield was obtained at a pH of about 7.5 (Fig. 6).

Effects of some enzymes on the yield of prostaglandin. It has been previously reported (ELIASSON 1957) that cobra venom (Naja naja) increases the yield of prostaglandin when the minced vesicular glands were incubated in two volumes of buffer. Since cobra venom contains rather

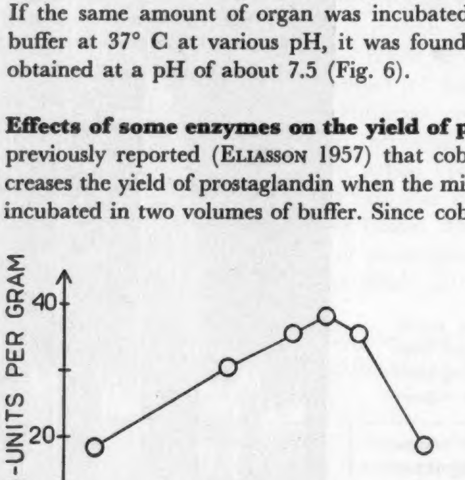

Fig. 5. Effect of temperature on the yield of prostaglandin (PG) from sheep's ground vesicular glands incubated for 60 minutes in 2 volumes of 0.15 M phosphate buffer at pH 7.5.

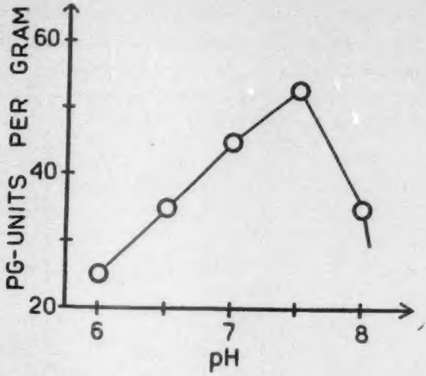

Fig. 6. Effect of pH on the yield of prostaglandin (PG) from sheep's ground vesicular glands incubated for 60 minutes in 2 volumes of 0.15 M phosphate buffer. Temperature 37° C.

high amounts of lecithinase A (SLOTTA 1953) the effect of this enzyme was studied in a series of experiments.

Different amounts of the enzyme was added to the buffer in which the ground vesicular glands were incubated (two volumes of buffer, v/w) for 60 minutes at 37° C. The results showed that 0.1 μg enzyme per ml buffer had a definite effect, 10 μg per ml giving a maximal yield corresponding to 100 units prostaglandin per gram organ. It was also noted that 1 μg lecithinase A was equivalent to 10 μg cobra venom (Naja naja), which agrees with the results by BOMAN (personal communication) that cobra venom contains about 10 per cent lecithinase A.

An increased yield was also obtained when some other enzymes with esterase activity (lipase, chymotrypsin, and trypsin)[1] were added to the incubation medium. Human blood serum esterases in Cohn's fractions IV-1 (phosphorylphosphatase) and IV-6 (cholinesterase) (AUGUSTINSSON and HEIMBÜRGER 1954), were found to be inactive in the doses used. Acetylcholinesterase from cobra venom (Hemachatus haemachates) showed an inhibitory effect (Table IV).

Glands which were incubated for 20 minutes in five volumes of buffer (10 ml) containing 0.5 to 3 ml human seminal plasma showed no increase in yield compared to the control samples. The same negative results were obtained if the vesicular glands were incubated with homogenates of the prostate and Cowper's glands of sheep or bull's vesicular glands.

The observation that the same high activity per gram organ could be obtained by increasing the amounts of buffer in which the organs were incubated (Table III) as with the addition of either cobra venom or lecithinase A to the incubation mixture using two volumes of buffer (v/w), suggested that lecithinase A might increase the initial velocity of the catalytic reaction by which prostaglandin is liberated. The validity of this interpretation was supported by the observations made in the

[1] Mann Research Lab. Inc. Lipase from calf gland, Trypsin 2 × cryst (50 % $MgSO_4$).

Table IV. Effect of different enzymes on the yield of prostaglandin from the sheep's ground vesicular glands incubated for 60 min in 2 volumes of 0.15 M phosphate buffer at pH 7.5. Temperature 37° C

Enzyme	Conc. per ml	Increase or decrease in activity per gram organ in per cent of control	Enzyme	Conc. per ml	Increase or decrease in activity per gram organ in per cent of control
Trypsin	50 μg	0	Cobra venom		
	200 μg	+ 30	(Naja naja)	10 μg	+ 100
Chymotrypsin	7.5 μg	0	Acetylcho-	10 μg	− 50
	30 μg	+ 40	linesterase	50 μg	− 50
Lipase	10 μg	0	Cohn's frac-	5 mg	0
	40 μg	+ 80	tion IV—1	10 mg	0
Lecithinase A	0.1 μg	+ 25	Cohn's frac-	5 mg	0
	1 μg	+ 100	tion IV—6	10 mg	0
	5 μg	+ 275			

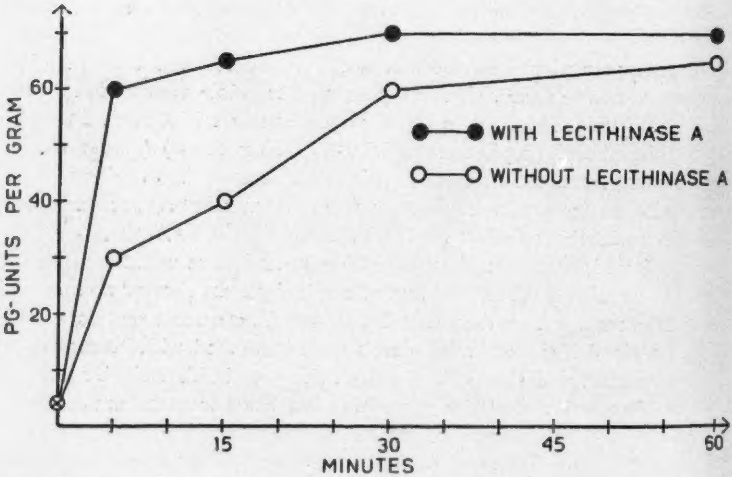

Fig. 7. Increase in yield of prostaglandin (PG) from sheep's ground vesicular glands incubated in 5 volumes of 0.15 M phosphate buffer at pH 7.5 with and without addition of 1 μg lecithinase A per ml. Temperature 37° C.

⊗ = non-incubated control.

experiments in which ground vesicular glands were incubated at 37° C for various periods of time in five volumes of buffer with and without 1 μg of lecithinase A per ml buffer. A representative experiment is illustrated in Fig. 7, which shows that lecithinase A does increase the initial reaction velocity. The activity per gram organ obtained after five minutes of incubation in buffer containing 1 μg of lecithinase A per ml is twice that in the organs incubated in buffer without the enzyme (Fig. 11). On the other hand almost the same activity was observed in both the control and test samples after 60 minutes of incubation. The blood pressure lowering effect as well as the effect observed on the isolated rat uterus of the two samples after 60 minutes of incubation are also of the same magnitude and character as is illustrated in Fig. 8, 9 and 10.

Effects of some enzyme inhibitors on the yield of prostaglandin. The minced vesicular glands were incubated for 20 minutes at 37° C in five volumes of buffer to which the inhibitors had been added in the concentrations indicated below. Of the enzyme inhibitors used, none was found to decrease the yield of prostaglandin.

The following inhibitors for esterases were used: diisopropyl fluorophosphate (DFP; 10^{-6} M and 5×10^{-6} M); diethyl p-nitrophenyl phosphate (E 600; 10^{-4} M and 5×10^{-4} M) and polyphloretin phosphate (10 to 500 μg/ml).

Similarly the following inhibitors for enzymes containing active

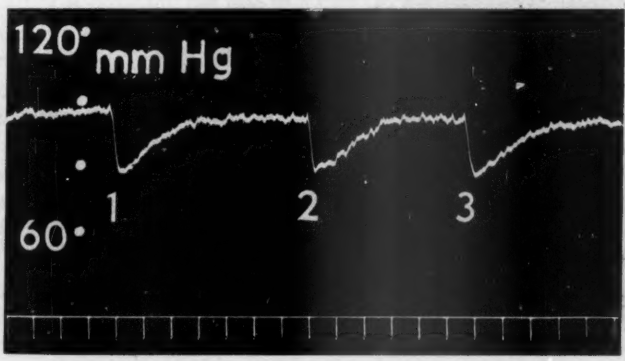

Fig. 8. Effect of i. v. injection of prostaglandin on the blood pressure of the rabbit anaesthetized with urethane and pretreated with atropine and an antihistamine.
Extracts from sheep's ground vesicular glands after 60 min of incubation with (3) and without (1) addition of 1 μg lecithinase A per ml. The amounts injected correspond to 40 mg tissue.
2 = 2 units standard. Time in min.

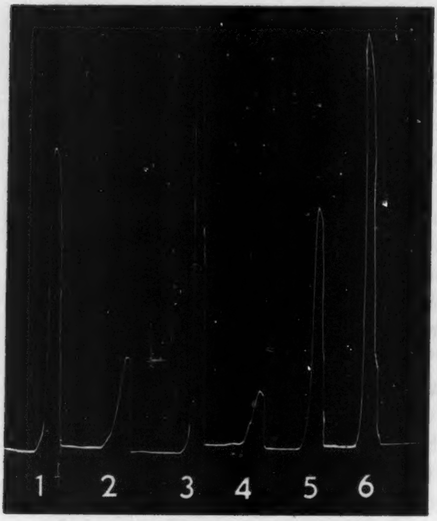

Fig. 9. Effect of prostaglandin extracted from sheep's ground vesicular glands on isolated rat uterus. Bath volume 3 ml. Temperature 30° C.

Extract from non-incubated organ: 1 = 1 unit, 2 = 0.5 unit, 3 = 1.5 units.

Extract from organ incubated for 60 min in 5 vol. of buffer (same extracts as at 1 in Fig. 8): 4 = 6 mg, 5 = 12 mg, 6 = 20 mg tissue.

Fig. 10. Effect of prostaglandin extracted from sheep's ground vesicular glands incubated with (1 and 4) and without (2 and 3) lecithinase A for 60 min on isolated rat uterus. Same extracts as in Fig. 8. Additions correspond to 6 and 10 mg tissue. Bath volume 3 ml. Temperature 30° C.

thiol-groups were used: iodoacetic acid (10^{-2} M) and p-chloromercuribenzoate (sodium salt, 10^{-3} M and 10^{-4} M).

Potassium cyanide (10^{-4} M) and the chelating agent EDTA (ethylenediaminetetraacetic acid) (10 to 260 mM) did not alter the yield of prostaglandin either.

Effects of various substances on the yield of prostaglandin. *Detergents:* The increase in the reaction velocity obtained with lecithinase A may be explained as a result of its esterase activity, or a disintegration of the cell membranes by the lysolecithin formed or a combination of these two. In order to study the effect of the rupturing of the cell membranes, the vesicular glands were incubated in a buffer containing Tween 80, cetylpyridinium bromide, or Teepol (Shell Oil Co.). Using 2 volumes of buffer there was a definite increase in activity after 60 minutes of incubation. When the organs were incubated for 20 minutes in 5 volumes of buffer there was a similar increase in yield indicating that the detergents used enhance the initial reaction velocity (Table V).

Compound 48/80 not only liberates histamine from perfused organs, but also another smooth muscle stimulating substance having the properties of what is named as a "slow reacting substance" (SRS, cf. page 55) (Paton 1951). It is known (Feldberg and Kellaway 1938) that cobra venom also liberates histamine and a SRS from perfused organs and it has been shown later by Högberg and Uvnäs (1957) that both 48/80 and lecithinase A, when tested on the mast cells in the rat mesentery, are potent histamine liberators. The effect of 48/80 on the yield of prostaglandin was therefore studied in eight experiments. In all cases there was a definite decrease in the yield of prostaglandin (Table V).

Adenosinetriphosphate (ATP) increased the yield of prostaglandin, when added in the concentration of 1—5 $\times$ 10^{-3}M. Addition of *sodium fluoride* (2—8 $\times$ 10^{-2} M) also increased the yield of prostaglandin (Table V). This effect is probably not due to a decreased rate of breakdown of the endogenous ATP (Nachmansohn and Machado 1943), since p-chloromercuribenzoate, which also inhibits adenosinetriphosphatase in the present investigation did not alter the prostaglandin yield.

Adrenaline, or *noradrenaline*, or *acetylcholine* (10 μg/ml) was added to the incubation mixture in two sets of experiments — one with preaddition of iproniazid (Marsilid®, Roche; 5 $\times$ 10^{-3} M) or neostigmine (Prostigmine®, Roche; 5 $\times$ 10^{-3} M) to prevent rapid breakdown of the added substance. Using 20 minutes incubation in five volumes of buffer, there was no alteration in yield.

Organic solvents: From the investigations by Stedman and Stedman (1937, 1939) it is known that the formation of acetylcholine in brain homogenates is enhanced by ether and chloroform, but inhibited by acetone. In this study addition of 0.5 ml of ether or chloroform to 10 ml of buffer in which the glands were incubated had a definite inhibitory effect. The same volume of acetone in 10 ml of buffer did not change the yield, while in a solution of 40 per cent acetone in buffer, the yield was decreased (Table V).

Table V. Effects of different substances on the yield of prostaglandin from the sheep's ground vesicular glands incubated for 20 min in 5 volumes of 0.15 M phosphate buffer at pH 7.5. Temperature 37° C

Substance	Concentration	Increase or decrease in activity per gram organ in per cent of control
Tween 80	0.01 %	+ 45
Cetylpyridinium bromide	0.01 M	+100
Teepol	0.07 %	+ 50
Ether	0.05 ml/ml	− 50
Chloroform	0.05 ml/ml	− 50
Acetone	0.05 ml/ml	0
Acetone	40 %	− 35
ATP	$1-5\times10^{-3}$ M	+130
NaF	$2-8\times10^{-3}$ M	+ 65
48/80	5 μg/ml	− 20
48/80	10 μg/ml	− 45
48/80	20 μg/ml	− 55

Yield of prostaglandin using acetone extraction under various conditions. Ground vesicular glands were extracted with two volumes of acetone at various pH using principally the same method as described on page 6 and the yield obtained is given in Table VI. On the other hand acetone extraction of freeze-dried organs in no case gave a yield of more than two units per gram wet weight.

Table VI. Yield of prostaglandin from sheep's ground vesicular glands extracted at room temperature with 2 volumes of acetone at various pH

Solution	pH	PG-units per gram	Solution	pH	PG-units per gram
Acetone + 3 N HCl	3	2	Acetone + 5 N NaOH	9	18
Acetone	7	30	Acetone + 5 N NaOH	12	4

One part of ground vesicular glands was also mixed with two volumes of acidified acetone (+ 3° C) and another part of the organ with two volumes of acetone (+ 3° C). After five minutes the samples were centrifuged for 15 minutes (+ 3° C; 3,000 *g*), the supernatant decanted and extracted for its content of prostaglandin. The sediments were mixed with two volumes of buffer at 37° C, shaken for 5 minutes at 37° C and extracted for their content of prostaglandin. In none of the six experiments were there more than three units per gram organ in the extracted

sediments incubated with buffer. Whether this effect of acetone treatment is due to a denaturation of the substrate, or the enzyme, or if one or both of these components have been removed by the acetone has not been further investigated.

II. Experiments with various organs

The accessory genital glands of ram were separately incubated in five volumes of buffer in order to test if there was an increase in yield of prostaglandin. The results (Table VII) show that formation of prostaglandin in vitro only occurs in the minced vesicular glands and ampulla ductus deferens. These two organs are also almost identical from histological and histochemical aspects (NICANDER, personal communication). Different samples from these two specimens also showed the same activity when assayed on the rabbit blood pressure and isolated rabbit jejunum.

Table VII. Yield of prostaglandin (PG) from ram's accessory genital glands incubated in 5 volumes of phosphate buffer at pH 7.5. Temperature 37° C

Organ	Incubation in minutes	PG-units per gram	Organ	Incubation in minutes	PG-units per gram
Amp. ductus def.	<1	10	Prostate glands	<1	1.5
	15	20–30		20	1.5
	60	30–40	Cowper's glands	<1	0.5
Vesicular glands	<1	10–15		60	0.5–1.0
	15	20–40			
	60	40–80			

In another experiment various other organs were extracted for prostaglandin-like activity after incubation at 37° C for 60 minutes in five volumes of buffer with and without the addition of 5 μg lecithinase A per ml. The following organs were used: dog's prostate gland, bull's vesicular glands, and sheep's lung, kidney and liver. The extracts were assayed on the rabbit blood pressure and isolated rabbit jejunum and compared with the prostaglandin standard.

By the isolated jejunum test a smooth muscle stimulating activity was found in all extracts. The activity did not, however, correspond to more than 6.5 prostaglandin units per gram, except for the kidney which gave a value of 13 units per gram. None of the tissue extracts had, however, a blood pressure lowering effect comparable to that on the rabbit jejunum. It is therefore concluded that the smooth muscle stimulating factors extracted from these organs were not prostaglandin.

III. Comparison of prostaglandin from non-incubated and incubated sheep's vesicular glands

Prostaglandin from the two sources were compared with regard to the *biological activities* on rabbit blood pressure, isolated rabbit jejunum, the isolated jejunum and uterus of rat, and guinea-pig ileum. On all but the guinea-pig ileum the two prostaglandin preparations showed the same activity, *i. e.* the index of discrimination was almost one (GADDUM 1955). Prostaglandin extracted from the incubated vesicular glands was about 2 to 10 times as active on guinea-pig ileum as on the rabbit jejunum, when compared with the prostaglandin standard. It was further recorded

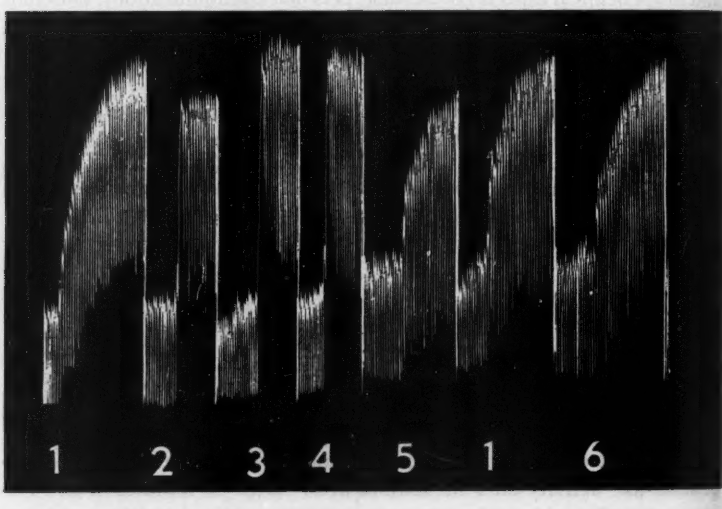

Fig. 11. Effect of prostaglandin extracts from various sources on isolated rabbit jejunum. Bath volume 15 ml.

Sheep's ground vesicular glands without incubation: 1 = 80 mg, 5 = 60 mg tissue.
Same incubated for 5 min in 5 vol. of buffer: 2 = 3 mg tissue.
Same incubated as in 2) with addition of 1 μg lecithinase A/ml buffer: 3 = 2 mg tissue.
Sheep's seminal fluid: 4 = 0.0035 ml.
Human seminal fluid: 6 = 0.0040 ml.

The record illustrates that on this test organ: a) prostaglandin extracted from sheep's incubated vesicular glands and sheep's seminal fluid have the same qualitative effect (2, 3, 4); b) prostaglandin from sheep's non-incubated vesicular glands and human seminal fluid has the same qualitative effect (1, 5, 6); c) the prostaglandin yield from organs incubated for 5 min with 1 μg lecithinase A per ml is about twice that from organs incubated without this addition (2, 3).

that prostaglandin from incubated organs usually gave rise to more rapid increase in tonus of the rabbit jejunum than did the standard preparation (Fig. 11).

The prostaglandin preparation obtained from sheep's seminal fluid, when the extraction method described on page 6 was used, was also found to be 2 to 10 times more active on guinea-pig ileum than on rabbit jejunum, *i. e.* when prostaglandin from incubated organs and sheep's seminal fluid were compared on the various test organs the index of discrimination, for these two prostaglandin preparations, was approximately one.

These results clearly indicate that biologically there is a difference between prostaglandin extracts from non-incubated vesicular glands in comparison with that from incubated ones. On the other hand incubation gives a prostaglandin preparation, whose biological actions on isolated rabbit jejunum, guinea-pig ileum and rat uterus are similar to that of prostaglandin extracted from sheep's seminal fluid.

Prostaglandin from incubated and non-incubated sheep's vesicular glands were also compared by *chromatography*, using a column of Whatman no. 1 cellulose powder and ethyl acetate/acetic acid/water (3 : 1 : 1) as eluant. The cellulose powder had been washed with 1 per cent oxalic acid followed by rinsing with distilled water and dried (KENNEDY and BARKER 1951). Seven grams of the powder was mixed with the solvent and poured into a glass tube with a diameter of 0.9 cm. The column was pressed by air to a length of 30 cm.

The prostaglandin solution was prepared as described on page 6. The phosphate buffer was then acidified to about pH 3 with concentrated hydrochloric acid and the prostaglandin extracted with three washes of $^1/_2$ volume of acidified ethyl acetate in accordance with the method described on page 6. The ethyl acetate was dried by adding excess of anhydrous sodium sulphate (ARMSTRONG, SHAW and WALL 1956), decanted and evaporated under reduced pressure. The residue was dissolved in 2 ml of the ethyl acetate/acetic acid/water mixture and added to the column. To the different collected effluent fractions was added 5 N NaOH to about pH 7 and the activity was extracted with three washes of $^1/_2$ volume phosphate buffer at pH 7.5.

Bioassay was performed on the blood pressure and isolated jejunum of the rabbit and the isolated guinea-pig ileum. The results are summarized in table VIII.

Although prostaglandin from both non-incubated and incubated organs showed the same Rf-value, there are differences in the results of the two experiments. Similar to what has been described above, the prostaglandin activity of the added solution from incubated organ was

Table VIII. Chromatography of prostaglandin (PG) on cellulose column

(length 30 cm, diameter 0.9 cm). Solvent: Ethylacetate/acetic acid/water (3 : 1 : 1)

PROSTAGLANDIN extracted from

Non-incubated organs				Incubated organs			
Effluent volumes ml	Activity per ml			Effluent volumes ml	Activity per ml		
	Rabbit blood pressure	Rabbit jejunum	Guinea-pig ileum		Rabbit blood pressure	Rabbit jejunum	Guinea-pig ileum
0—10.1	0	0	0	0—9.4	0	0	0
14.1	0	0	15	11.8	0	16	6
16.0	120	110	105	14.2	35	35	25
17.7	250	240	250	16.3	290	290	570
19.3	150	160	150	18.6	300	310	300
20.9	30	15	17	20.9	9	13	15
22.4	0	0	5	23.2	0	0	15
22.4—80.0	0	0	0	23.2—50.0	0	0	0
PG added; units	1 400	1 400	1 400	PG added; units	2 000	2 000	3 600
PG recovered, total; units	920	897	959	PG recovered, total; units	1 400	1 475	2 050
Recovery; per cent	66	64	69	Recovery; per cent	70	74	70

higher when tested on guinea-pig ileum than on the blood pressure and isolated jejunum of the rabbit. The factor(s) responsible for this enhanced activity can be distinctly localized to the effluent fraction which contains the peak prostaglandin activity. The activity of this fraction like that of the added extracts, when tested on the guinea-pig ileum, was about twice the activity obtained on the isolated rabbit jejunum. This sharp localization of the new factor seems to offer a possibility for further study of its nature.

The results of the two experiments also show another difference between prostaglandin extracts from non-incubated and incubated organs. The total activity of the extract from incubated organ seems to consist not only of prostaglandin but also of a factor which is active on the isolated intestinal preparations but does not change the rabbit blood pressure. That this actually should be the case is not unlikely, since most tissues seem to contain an "unspecific" activity of this kind (VOGT 1958 b; cf. above). It corresponded to about 2 per cent of the total activity in the sample, and therefore did not interfere with the bioassay of the original extract.

IV. Comments and conclusions

The results obtained when the sheep's ground vesicular glands were incubated under different conditions (various temperature, addition of enzymes, etc.) have shown that prostaglandin is present in the tissue in an inactive form and that it can be rapidly split off in amounts which correspond to those found in the seminal fluid. The yield of prostaglandin obtained with incubation at various pH-values as well as the experiments performed with acetone extraction of sheep's ground vesicular glands under various conditions clearly indicate that prostaglandin can not be bound in the tissue with an electrostatic (salt) linkage, since this ought to dissociate in acid or basic solutions at least to the same extent as in neutral solution.

Since prostaglandin presumably is a carboxylic acid and since enzymes like lipase and trypsin increase the yield of prostaglandin, it seems most likely that prostaglandin is bound in the tissue by an ester-linkage.

The physiological process behind the liberation of prostaglandin is at present not known. The experiments performed with various lytic agents suggest that the inactive prostaglandin complex and an enzyme for liberation of the active prostaglandin are present in the same cellular structures, although probably separated by cell membranes. The high velocity of liberation also suggests that the enzyme and substrate are located in close proximity to each other.

The increase in yield obtained by addition of detergents to the incubation mixture is most likely due to rupturing of the cell membranes, although other actions can not be excluded. Thus it is known that detergents can form stable micelles and hence cause physical dispersion of water insoluble material (Colowick and Kaplan 1955, p. 35). Whether the action of lecithinase A is due mainly to its formation of a lytic substance (lysolecithin) or to its esterase activity can not be stated with certainty.

From the results obtained it can be concluded that the increase in activity is specific and not due to the widely distributed slow reacting substances (SRS) demonstrated by Feldberg and Kellaway (1938), Kellaway and Trethewie (1940), Paton (1951), Brocklehurst (1953, 1955, 1956), and Högberg and Uvnäs (1957). The inhibiting effect of compound 48/80 also supports this conclusion. The reason why 48/80 actually decreases the liberation of prostaglandin is not known, but it may be possible that in the many catalytic processes in the crude incubation mixture, factors are formed which inhibit the prostaglandin liberation and that the formation of such inhibiting factors can be enhanced by 48/80.

The biological comparison showed that prostaglandin from the incubated organs was 2 to 10 times as active on the isolated guinea-pig ileum as on the rabbit jejunum compared with the standard. It therefore appears that incubation releases a factor, which either differs from that responsible for most of the activity in extracts of non-incubated organs, or potentiates the effect of prostaglandin on the guinea-pig ileum.

In the chromatography, using a column of Whatman no. 1 cellulose powder, it was found that the new principle could be distinctly located to that effluent fraction which contained the peak prostaglandin activity (Table VIII). The sharp localization and the good recovery seem to facilitate further studies on the chemical and biological characteristics of this factor.

Prostaglandin extracts from sheep's seminal fluid not only gave the same qualitative response on rabbit jejunum as prostaglandin from incubated vesicular glands (Fig. 11), but was also 2 to 10 times as active on the isolated guinea-pig ileum as on the rabbit jejunum, when compared with the standard. It is therefore clear that prostaglandin extracts obtained from incubated sheep's vesicular glands possesses biological characteristics similar to those found in extracts from sheep's seminal fluid. It does not appear unlikely that the similarity in action between the two preparations depends upon the same factor.

In an earlier publication it was shown that prostaglandin extracted by acid alcohol and ether from human seminal fluid and from sheep's non-incubated vesicular glands on a variety of test organs including guinea-

pig ileum had the same biological effects (ELIASSON 1957). The response pattern of the isolated rabbit jejunum following addition of prostaglandin from these two sources was also similar as is illustrated in Fig. 11. It therefore appears that the difference in biological actions between the prostaglandin extracts of human and of sheep's semen is of the same kind as that between the prostaglandin extracts of non-incubated and of incubated sheep's vesicular glands described above.

5. EFFECT OF PROSTAGLANDIN ON THE MOTILITY OF NON-PREGNANT UTERUS IN VITRO

That human seminal fluid alters the motility of isolated strips of human nonpregnant uterus was first shown by Kurzrok and Lieb (1930) and Cockrill, Miller and Kurzrok (1935), who measured the effects of different samples of seminal fluid on isolated tissue strips cut parallel with the external muscle fibres in more than 400 corpora uteri preparations. The normal response of the uterus preparation was that of relaxation but some specimens responded with increased motility and tonus. The seminal fluid from about 13 per cent of the examined men (75 cases) gave rise to increased tonus in many of the preparations. It was also observed that those uterine strips which were hyperactive to all seminal fluids were obtained from patients giving a history of prolonged or complete infertility. It has been suggested that this reaction towards semen may be an important factor for the infertility in these patients.

The effect of prostaglandin extracted from seminal fluid with alcohol and ether were examined by Euler (1936) on isolated strips from uterus of man and various animals. With human uterus an increase in amplitude and in some cases also in frequency of the contractions was observed. In one case the strip consisted of longitudinal fibres, the remainder consisted of strips taken from the circular fibres arranged around myomatous nodes. The response to prostaglandin was similar in both cases. Prostaglandin also caused contractions and increased motility of the uteri from the animals investigated. In a preparation from a cow in early pregnancy, however, prostaglandin caused an inhibition.

The following investigation deals with the effect of prostaglandin on isolated strips from human uterus with simultaneous recording of motility of tissue cut from different anatomical parts of the uterus. The effect of prostaglandin on isolated uterine preparations from rabbits and rats pretreated with ovarian hormones will also be reported.

Methods

Muscle strips from human uterus were taken from preparations obtained after hysterectomy in patients with uterine fibroids. The strips were cut longitudinally from the corpus and transversally from the isthmus and (when possible) the cervix according to the method described by Sandberg *et al.* (1957).[1] The strips were immersed in ice cold Ringer solution and used within one hour.

[1] The uterine preparations were kindly supplied by Dr. L. Lindgren, the Sabbatsberg's Hospital, and Dr. N. Posse, the Allmänna Barnbördshuset, Stockholm.

The strips from corpus, isthmus and cervix measuring about 20 × 3 × 3 mm were immersed together in a 220 ml organ bath containing Tyrode solution (37° C) modified according to Genell (1937), aerated with 5 per cent carbon dioxide in oxygen and the motility of the various strips registered simultaneously on a smoked drum. The recording was in principle isometric, the amplification of the movement being 32 : 1. The Tyrode solution had the following composition: 0.8 per cent NaCl; 0.042 per cent KCl; 0.024 per cent $CaCl_2$; 0.0005 per cent $MgCl_2 \cdot 6\ H_2O$; 0.1 per cent $NaHCO_3$ and 0.05 per cent glucose.

The *rabbits* were divided in four groups of four according to the pretreatment. *Group 1* being in natural oestrus; *Group 2* ovarectomized and given 10—15 μg stilbol in peanut oil per kg intramuscularly for three days; *Group 3* ovarectomized and given 10 μg stilbol and 1 mg progesterone in peanut oil per kg intramuscularly for three days; and *Group 4* 10 μg stilbol for three days and then made pseudo-pregnant by intravenous injections of 100—200 units of gonadotrophin (Gonadex®, Leo). The animals in group 4 were used on the fourth day after the last injection, all the others being used the day after the last injection. Ovarectomy was usually performed two weeks before the experiments.

The animals were anaesthetized with urethane and bled to death, the uterus removed and immersed in 15 ml Tyrode solution (37° C) aerated with 6.5 per cent carbon dioxide in oxygen. The movements were recorded in accordance with the general methods.

The *rats* were spayed two to three weeks before the experiments and given the following pretreatment: *Group 1* (4 animals) was given 2 μg oestradiol-17 β (Schering) in propylene glycol for three days; *Group 2* (7 animals) was given the same dose of oestradiol-17 β and in addition 2 mg progesterone (in peanut oil) for three days; and *Group 3* (2 animals) 2 μg oestradiol-17 β and 2 mg progesterone, and 0.3 mg relaxin (150 GPU/mg) in 1 per cent L 390[1] for the same period of time as the others. All injections were given subcutaneously. The animals were killed by a blow on the head, the uterus removed and one horn suspended in a 3 ml organ bath containing ordinary Tyrode solution at 37° C and aerated with 6.5 per cent carbon dioxide in oxygen.

Prostaglandin extracted from human seminal fluid as described on page 6 and containing 50 units per ml was used in the experiments with human uterus. In the other experiments prostaglandin standard (20 units/ml) was used.

Results

Human uterus. In three out of five cases prostaglandin caused a marked inhibition of the spontaneous motility of uterine preparations obtained from fertile patients with the endometrium in late proliferative stage, when given in doses of about 0.002 to 0.01 units per ml bath fluid. In the other two cases small doses gave an increase in amplitude of the contractions and also seemed to synchronize the activity of the different muscles in the strips, but when the dose was increased the reaction changed

[1] Benzopurpurine 4-B (respository suspension); kindly supplied by Dr. R. L. Kroc, Warner—Chilcott Lab, Morris Plains, N. J.

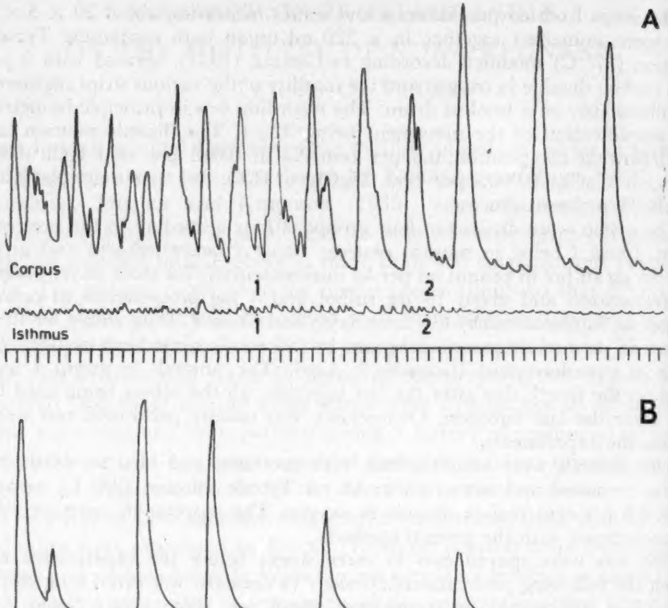

Fig. 12. Effect of prostaglandin and native human seminal fluid on isolated strips from corpus and isthmus of human non-pregnant uterus in late proliferatory stage. Bath volume 220 ml. Between records 30 to 60 min interval. Time in min.

Prostaglandin extracted from human seminal fluid: 1 = 0.5 unit, 2 = 1 unit, 3 = 2.5 units.

Native human seminal fluid: 4 = 0.02 ml.

to inhibition (Fig. 12). This observation may offer an explanation to the difference in results previously reported by Kurzrok and co-workers (1930, 1935) and Euler (1936).

The uterine strips from two patients who had passed the menopause, did not respond to prostaglandin and the same was found to be the case with a preparation from a patient with metropathia hemorrhagica cystica.

When the quantitative response to prostaglandin of strips from corpus and isthmus were compared, the latter was more sensitive. Strips from cervix uteri were obtained only in a few cases and showed no spontaneous activity. No definite change of the tone was observed after addition of prostaglandin.

Seminal fluid changed the motility pattern in the same way as did prostaglandin. In Fig. 12 the effect of a small amount of seminal fluid (C) is compared with that of prostaglandin (A and B) on the same preparation. Fig. 13 illustrates that 7 units of prostaglandin per 100 ml bath fluid has the same dramatic effect upon the tonus and motility of the isolated uterine strip as was generally recorded by Cockrill, Miller and Kurzrok (1935) after addition of 1 ml seminal fluid per 100 ml bath fluid to similar preparations. The data given on page 10 and the results by Asplund (1947 a) have shown that the average value of prostaglandin content in seminal fluid of infertile men is about 10 units per ml. The results in Fig. 13 with a prostaglandin preparation and the results by Cockrill, Miller and Kurzrok (1935) therefore seem to be directly comparable.

[**Rabbit's uterus.** Uteri from rabbits in normal or artificial oestrus (groups 1 and 2) usually responded to prostaglandin with a decrease

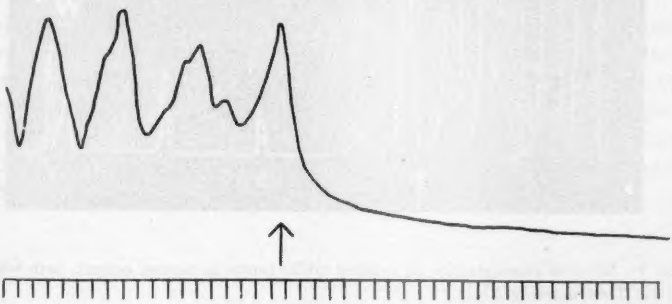

Fig. 13. Effect of prostaglandin on an isolated strip from corpus of non-pregnant human uterus obtained 2 to 4 days after a normal ovulation. Bath volume 220 ml. Time in min. At arrow 15 units prostaglandin extracted from human seminal fluid.

in activity (Fig. 14 and 15). On the other hand increased activity was also recorded, as is illustrated in Fig. 25 (p. 52). Similarly the isolated uteri from oestrogen and progesterone treated rabbits showed both types of response towards prostaglandin (Fig. 16).

It has not been possible to find any correlation in these three groups between the type of response and the experimental conditions. Thus for example there was no difference in the histological picture[1] of the two uteri used in the experiments illustrated in Fig. 14 and 25. Moreover, the two animals used in the experiments illustrated with Fig. 16, were of the same weight, ovarectomized on the same day, given the same hormonal pretreatment, and the isolated uterus tested under the same experimental conditions. Despite this prostaglandin in one of the cases caused inhibition, in the other stimulation.

Uterine preparations from the pseudo-pregnant rabbits always — but in some cases after a latency period of minutes — responded with an increased tonus and motility towards prostaglandin in doses of 1 to 4 units (Fig. 17). The threshold dose of prostaglandin in this group was

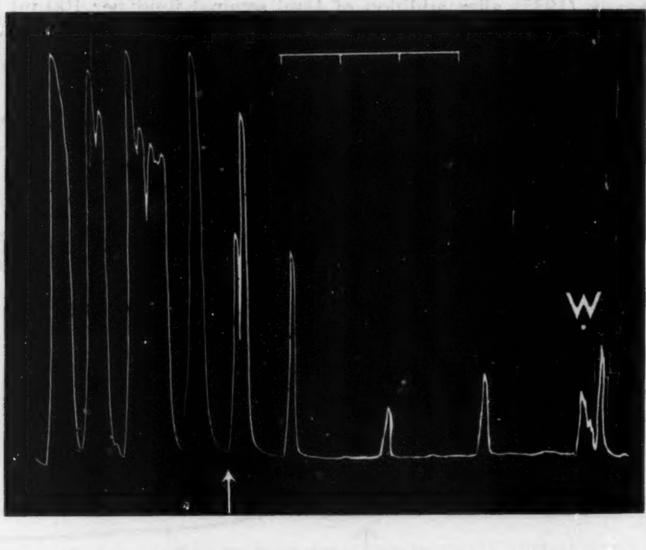

Fig. 14. Effect of prostaglandin on isolated rabbit uterus in normal oestrus. Bath volume 15 ml. Time marking 2 min.

At arrow 0.1 unit prostaglandin standard. w = wash.

[1] Kindly performed by Dr. B. FREDRIKSSON, Department of Histology, Karolinska Institutet, Stockholm.

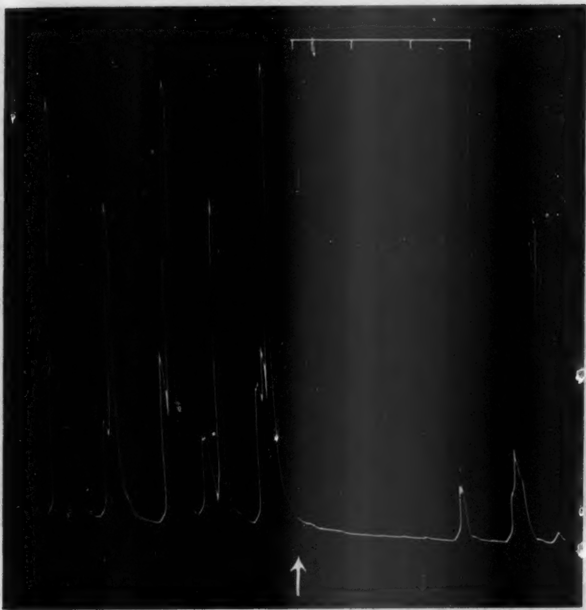

Fig. 15. Effect of prostaglandin on isolated rabbit uterus pretreated with 50 μg stilbol for 3 days. Bath volume 15 ml. Time marking 2 min.
At arrow 0.1 unit prostaglandin standard.

about ten times higher than in the other three groups, *i. e.* about 0,07 unit per ml bath fluid.

Rat's uterus. The rat's uterus was less sensitive towards prostaglandin than the human and rabbit's uteri. The response towards prostaglandin was in all cases an increase in tonus and usually also in amplitude and frequency of the spontaneous contractions. Relaxin, which was given to one of the groups since it is known that this hormone is produced in the rabbit's uterus under influence of progesterone (HISAW and ZARROW 1950), did not alter the response towards prostaglandin (Fig. 18).

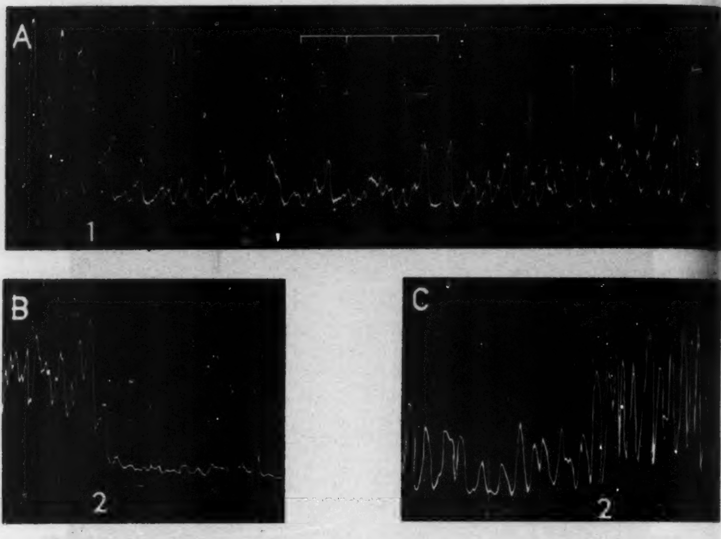

Fig. 16. Variation in effects of prostaglandin on isolated rabbit uterus. The two animals used were ovarectomized and pretreated with 30 μg stilbol and 3 mg progesterone for 3 days. Bath volume 15 ml. Time marking 2 min. A and B from the same preparation.

1 = 0.1 unit, 2 = 0.4 unit prostaglandin standard.

Fig. 17. Effect of prostaglandin on a pseudo-pregnant rabbit's isolated uterus. Bath volume 15 ml. Time marking 2 min.

At arrow 2 units prostaglandin standard.

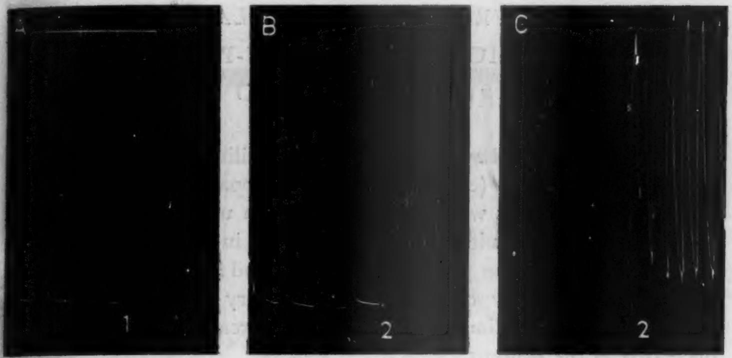

Fig. 18. Effect of prostaglandin on isolated rat uterus. Bath volume 3 ml. Time marking 2 min. The ovarectomized animals were given different pretreatment.
A: 2 μg oestradiol for 3 days.
B: 2 μg oestradiol and 2 mg progesterone for 3 days.
C: 2 μg oestradiol, 2 mg progesterone and 45 GPU relaxin for 3 days.
1 = 1 unit, 2 = 2 units prostaglandin standard.

6. EFFECT OF PROSTAGLANDIN ON THE MOTILITY OF NON-PREGNANT UTERUS IN VIVO

Several investigators have measured the motility of the nonpregnant human uterus in vivo (cf. Posse 1958), but apparently only one report (Karlson 1949) deals with the effect of semen upon this motility. This author measured simultaneously the activity in corpus, isthmus, and cervix uteri, with three pressure transducers and found that seminal fluid increased the activity during the proliferatory and secretory stages. At the time of ovulation the activity was increased in the corpus and decreased in the isthmus.

The effect of human seminal fluid and prostaglandin on the motility of the rabbit uterus in vivo was studied by Asplund (1947 b), who found prostaglandin to increase the motility of the uterus and decrease the tonus of the abdominal tubal ostium.

In the present investigation the influence of prostaglandin on the motility in vivo of the nonpregnant uterus of rabbits and rats has been studied. The results obtained on the human uterus in vivo will be published elsewhere (Eliasson and Posse 1959), although some results from this study will be mentioned in the general discussion.

Methods

Rabbits, weighing 2.5—3.5 kg were divided into four groups according to pretreatment. Ovarectomy was usually performed two weeks before the in vivo experiments. All injections of stilbol and progesterone (in peanut oil) were given intramuscularly and the animals used the day after the last injection.

The animals were anaesthetized with urethane (1.5 g/kg) intravenously, atropine (2 mg/kg) and phenbenzamine (1 mg/kg) were given subcutaneously. The blood pressure was recorded from the common carotid artery with a rubber membrane manometer on a photographic film. The uterus was exposed by a low midline incision, and a rubber catheter inserted up the vagina and 3 cm into one of the uterine horns. The catheter was kept in position by ligatures round the uterus, particular care being taken not to interfere with the circulation, and at the introitus vaginae. The Fallopian tube was ligatured to prevent leakage. The recording system and uterine cavity were filled with Ringer solution to an initial pressure of + 2 or + 4 cm H_2O.

In 6 of the 19 experiments the other horn was used for recording the isometric contractions by placing it in a plexiglass chamber and fixing the distal and proximal ends with steel hooks. From the mid part of the uterus a thread was connected to the lever of a tambour joined to a strain gauge pressure transducer (Fig. 19). Since the fluid displacement in this

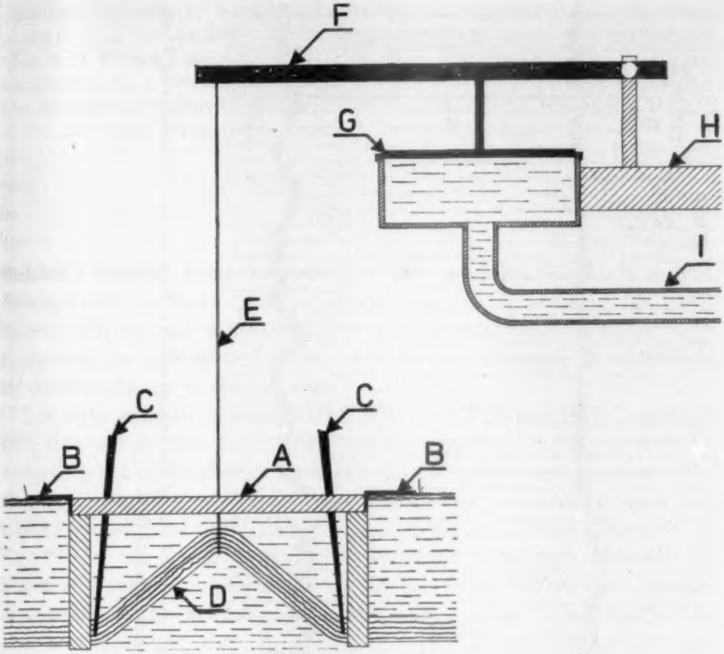

Fig. 19. Illustration of the principle used for the recording of the isometric contractions of the rabbit's uterus in vivo. The plexiglass chamber (A) was sewn to the abdominal skin (B) and the uterus kept in position by two steel hooks (C). The uterine contractions are transferred via a thread (E) and lever (F) to the thin metal plate (G), resting on a rubber membrane. The tambour is held in position by a rod (H), and connected by the tube (I) to a pressure transducer by water transmission.

system is almost zero the isometric contraction of the uterus is measured. The transducer was coupled to an electromanometer (Elema, Sweden) and the activity recorded on a photographic film. The position of the tambour could be adjusted with a screw. After 10—15 minutes the uterus was stretched by elevating the receptor compartment until there was a small increase in tension, indicating that the "resting level" was reached (Csapo 1954). The intrauterine pressure was in these cases recorded from the other horn by a sensitive rubber membrane manometer.

Prostaglandin (50 units per ml) extracted from human seminal fluid as described on page 6 was used. All injections were given into the external jugular or femoral vein.

The spayed *rats* were divided into two groups, one was given 2 μg oestradiol-17 β (Schering) in propylene glycol, the other 2 μg oestradiol-17 β + 2 mg progesterone in peanut oil subcutaneously on three succeeding days and the animals used on the fourth day. The animals were anaesthetized with sub-

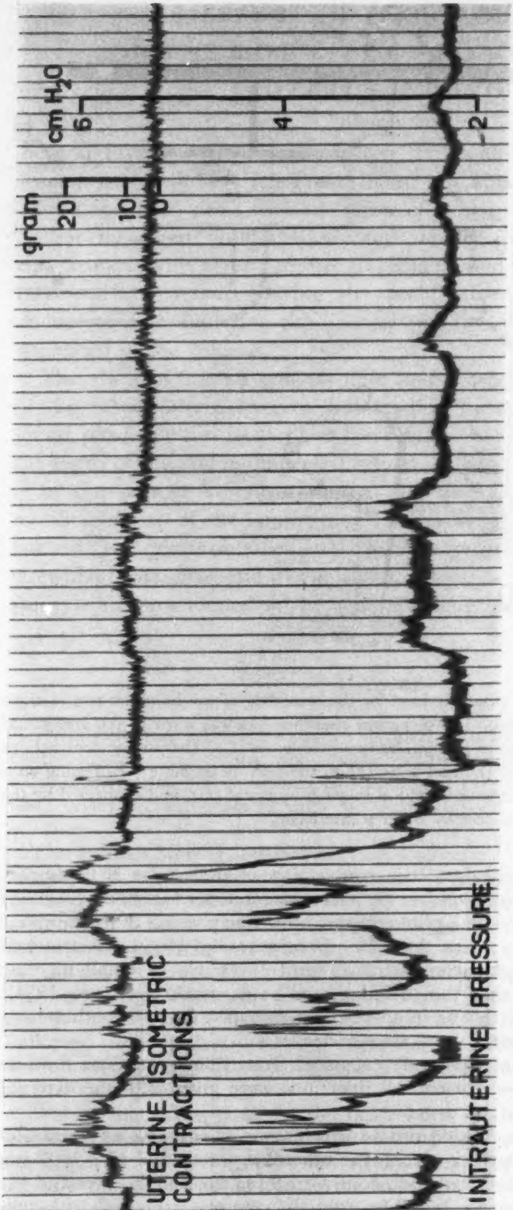

Fig. 20. The inhibitory effect of prostaglandin on the motility of an ovarectomized, stilbol-treated rabbit's uterus in vivo. Time marking 30 sec. At signal i. v. injection of 2 units prostaglandin extracted from human seminal fluid.

cutaneous injection of Nembutal® (Abbott; 4.5 mg/100 gram), the uterus exposed by a low midline incision and one of the horns was cannulated, filled with Ringer's solution at + 2 cm H_2O and connected to an electromanometer via a strain gauge pressure transducer. The prostaglandin solution as mentioned above contained 50 units per ml, and the injections (0.02 to 0.1 ml) were given intramuscularly in the fore legs.

Results

Rabbit's uterus. *Group 1* consisted of five untreated animals in oestrus. Prostaglandin in doses of 4 to 10 units gave a decrease in the spontaneous activity and tonus in two of the animals. One responded with an increase in activity and tonus, while in the remaining two there was no definite change of the activity.

The eight animals in *group 2* were ovarectomized and then pretreated with 10 to 30 μg stilbol per kg for various periods of time (3 to 8 days). Animals no. 1 and 2 did not show any change in the spontaneous motility after injection of 2 to 5 units prostaglandin; in no. 3 and 4 there was a definite inhibition of the motility, one of the cases being illustrated in Fig. 20. In no. 5 and 6 there was an initial stimulation followed by a decreased activity as is illustrated in Fig. 21. In no. 7 there was a definite stimulation of the activity, while in no. 8, 2 units prostaglandin first gave a stimulation, then thirty minutes later it was less active, and after another two hours it caused a weak inhibition of the activity (Fig. 22).

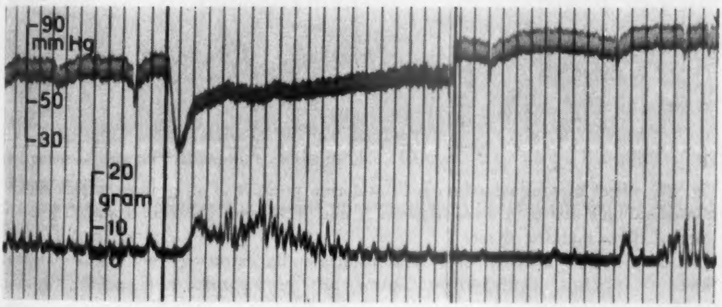

Fig. 21. Effect of prostaglandin on the blood pressure and the isometric contractions of an ovarectomized, stilbol-treated rabbit's uterus in vivo. Time marking 30 sec.
First signal: i. v. injection of 2 units prostaglandin extracted from human seminal fluid.
Second signal: the camera stopped for 10 min.

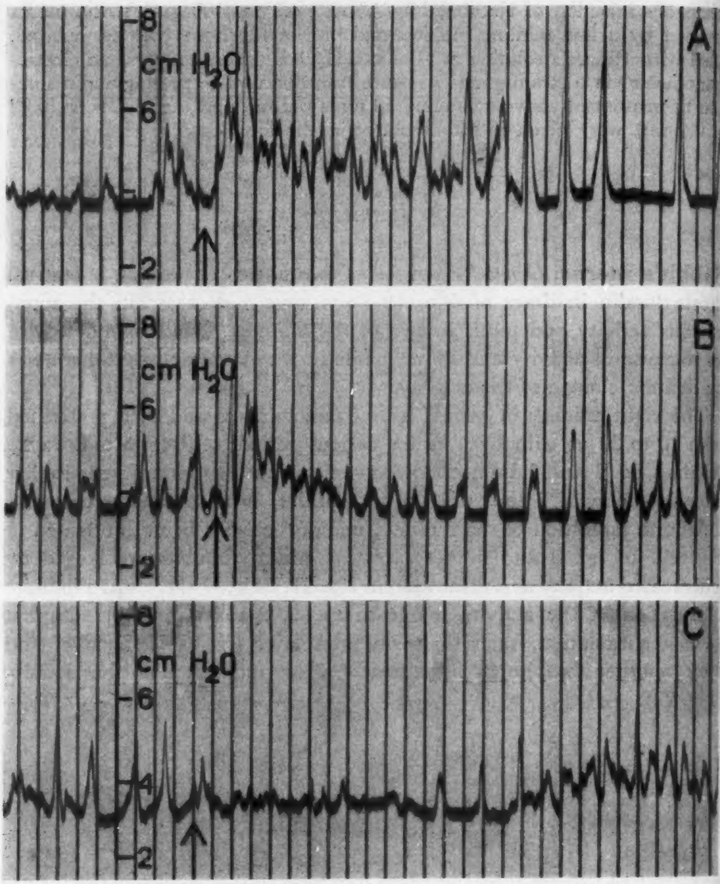

Fig. 22. Effect of prostaglandin on intrauterine pressure of an ovarectomized, stilbol-treated rabbit's uterus in vivo. Between A and B 30 min, between B and C 2 hours interval. Time marking 30 sec.

At arrows i. v. injection of 2 units prostaglandin extracted from human seminal fluid.

It was not possible to find any correlation between the type of response to prostaglandin and the experimental conditions, *e. g.* hormone pretreatment, blood pressure level, initial intrauterine pressure, etc.

Group 3 included four ovarectomized rabbits pretreated with 10 μg stilbol and 1 mg progesterone per kg for three days. Three of these animals

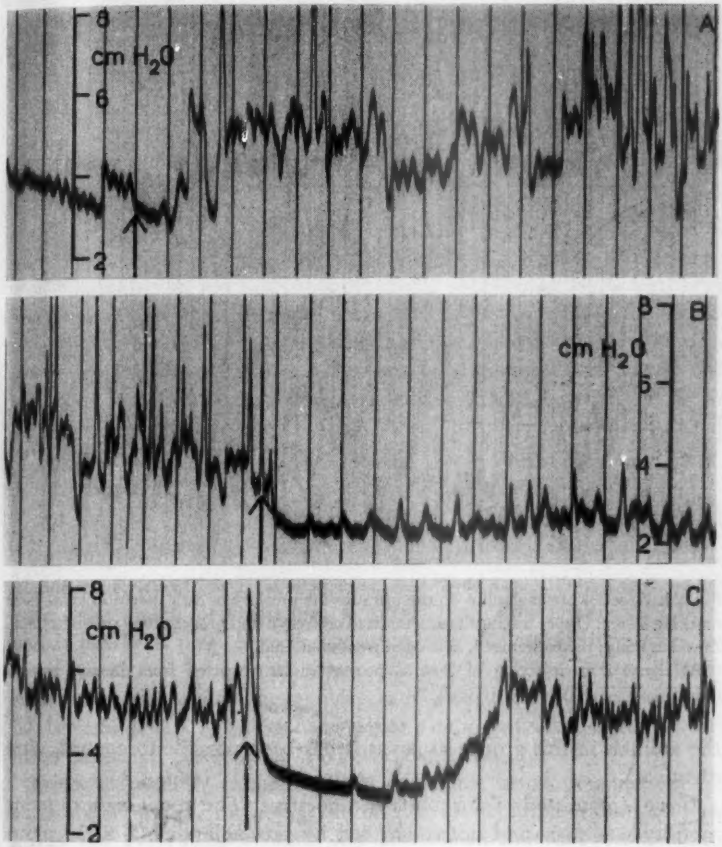

Fig. 23. Effect of prostaglandin on the intrauterine pressure of an ovarectomized, stilbol and progesterone treated rabbit's uterus in vivo. Time marking 1 min. Between records 30 min interval.

At arrows i. v. injection of 2.5 units prostaglandin extracted from human seminal fluid.

did not show any change of the weak spontaneous uterine motility after injection of 2.5 to 4 units prostaglandin. In the fourth animal 2.5 units prostaglandin first caused a marked increase in the tonus and motility, the following injection producing a marked decrease in tonus and motility. When the initial intrauterine pressure in this case was increased from + 4 to + 8 cm H_2O the inhibitory effect of prostaglandin was more pronounced (Fig. 23). The various experimental conditions for

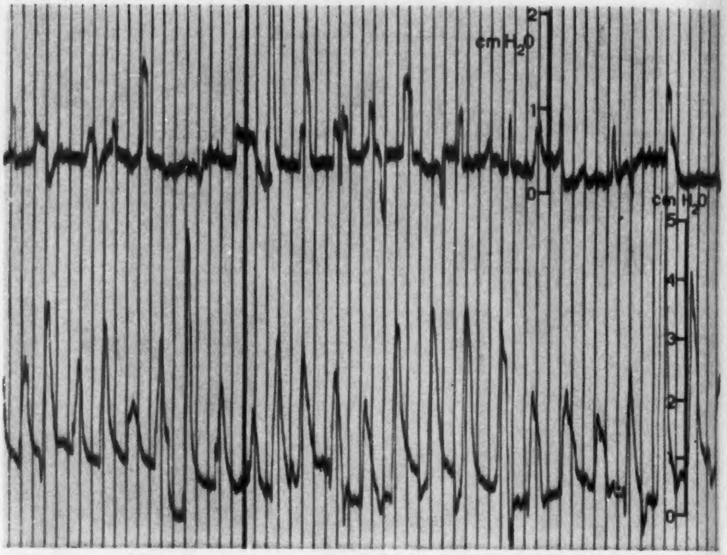

Fig. 24. Effect of prostaglandin on the intrauterine pressure of rat's uterus in vivo. Time marking 30 sec. Upper tracing: ovarectomized oestrogen and progesterone treated animal. Lower tracing: ovarectomized, oestrogen treated animal.

At signal i. m. injection of 5 units prostaglandin extracted from human seminal fluid.

the animals in this group, so far as it has been possible to control, were identical.

Group 4 consisted of 2 rabbits in anoestrus. The spontaneous uterine motility was poor and not influenced by prostaglandin (2 to 10 units) or oxytocin (0.01 to 0.2 I. U.).

In the experiments where both the isometric uterine contractions and intrauterine pressure were recorded, no qualitative difference was found between the two methods.

Rat's uterus. In nine oestrogen pretreated animals prostaglandin had no or only a short, weak and probably unspecific stimulating action on the uterus in doses up to 5 units. Also in eight oestrogen and progesterone pretreated animals there was no change in the uterine activity to a maximum of 5 units prostaglandin (Fig. 24). From other experiments intramuscular injections of these doses of prostaglandin are known to produce a pronounced lowering of the rat's blood pressure.

Prostaglandin in relation to other naturally occurring smooth muscle stimulating organic acids

Prostaglandin was the first smooth muscle stimulating factor described which possessed the chemical properties of an organic acid (EULER 1935 b, 1936, 1939). Several other substances with similar chemical and biological characteristics have later been demonstrated (for review see VOGT 1958 b). It is pertinent to clarify if and how prostaglandin can be separated from these other factors.

PROSTAGLANDIN E AND F

Two compounds have recently been isolated from sheep's vesicular glands and crystallized (BERGSTRÖM and SJÖVALL 1957, 1959). They have been called prostaglandin E (PGE) and prostaglandin F (PGF) and it has been possible to demonstrate clear biological differences between these two compounds (BERGSTRÖM *et al.* 1959 b). On different smooth muscle organs the activity ratio varied from 0.1 to more than 50. Injected i. v. 0.5 μg PGE has a definite depressor effect on the rabbit's blood pressure, while 10 μg PGF had no effect.

From the activity ratios obtained on rabbit blood pressure and on various test organs, including rabbit and rat jejunum, rabbit and rat uterus and guinea-pig ileum it was also shown that the prostaglandin preparation, used as standard in this investigation, neither could be identical with PGE, nor with PGF. This is further substantiated by the results obtained on the rabbit's isolated uterus in normal oestrus illustrated in Fig. 25. In this preparation prostaglandin stimulated the activity and did not show tachyphylaxis, while PGE[1] and PGF[1] not only had an inhibitory action on the motility, but also showed a marked tachyphylaxis.

The possibility that prostaglandin standard preparation might be a mixture of PGE and PGF was discussed in the paper by BERGSTRÖM *et al.* (1959 b). From the results obtained it was concluded, however, that a combination of only PGE and PGF could not explain the biological

[1] Kindly supplied by professor S. BERGSTRÖM, Dept. of Chemistry, Karolinska Institutet, Stockholm.

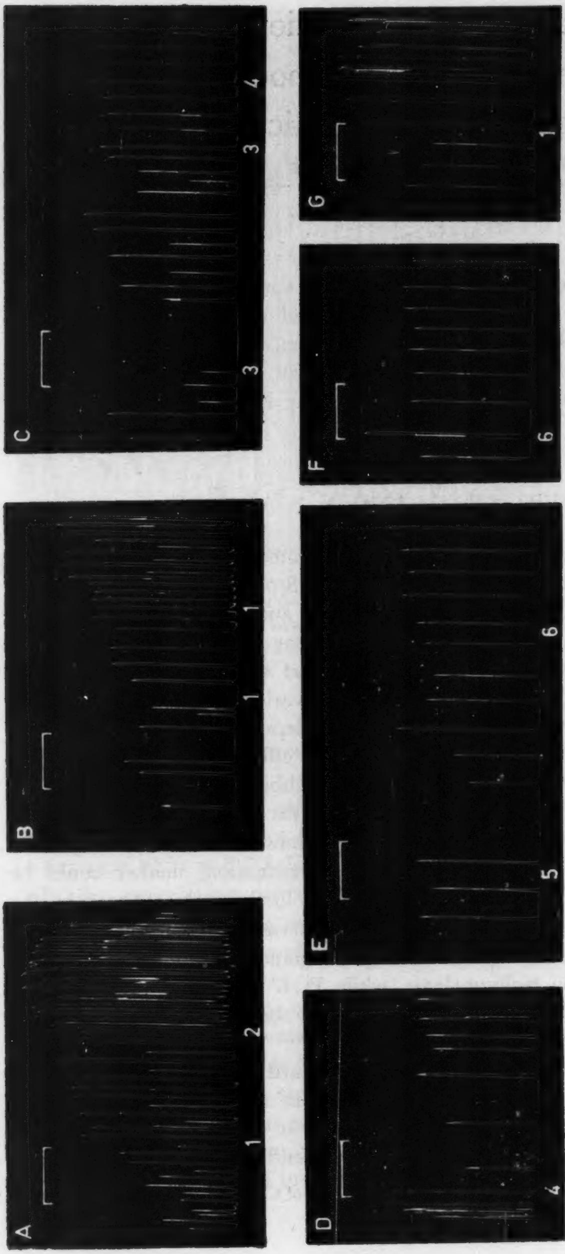

Fig. 25. Effect of prostaglandin, PGE and PGF on the isolated uterus from a rabbit in normal oestrus. Bath volume 15 ml. Time marking 5 min. Prostaglandin has a stimulating action (A, B and G) ,and shows no tachyphylaxis. 1 = 0.1 unit. 2 = 0.2 unit, PGE has an inhibitory action (C and D), which, however, is lacking if the substance is again added without washing. 3 = 0.5 μg, 4 = 1 μg PGE. PGF like PGE shows an inhibitory action as well as tachyphylaxis (E and F). 5 = 0.5 μg, 6 = 1 μg PGF.

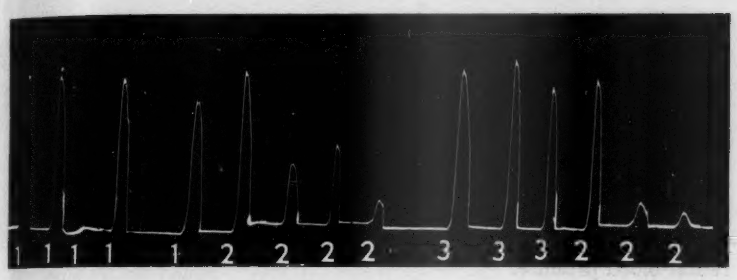

Fig. 26. Effects of various prostaglandin preparations on isolated rat uterus. Bath volume 3 ml. Temperature 30° C.
Prostaglandin extracted from sheep's semen: 1 = 0.085 ml semen.
Prostaglandin E (PGE): 2 = 10 μg.
Prostaglandin extracted from sheep's incubated vesicular glands: 3 = 20 mg tissue.

actions of this prostaglandin preparation. From the experimental data we could neither decide whether prostaglandin is a separate factor, or a combination of several active principles.

Prostaglandin from incubated sheep's vesicular glands in some biological aspects differ from the prostaglandin standard (page 30—31). Some of the biological actions of the former preparation are similar to those of PGE, *e. g.* it often causes a rapid increase in tonus of the isolated rabbit jejunum and is more active on the isolated guinea-pig ileum than on rabbit jejunum when compared with prostaglandin standard (BERGSTRÖM *et al.* 1959 b). However, PGE often shows an irregular effect on the isolated rat uterus, and sometimes also a marked tachyphylaxis and can therefore in some test preparations be separated from prostaglandin extracted from incubated sheep's vesicular glands and from sheep's seminal fluid. The difference is illustrated in Fig. 26, where the isolated rat uterus shows a fairly regular response towards prostaglandin both from the sheep's incubated vesicular glands and from sheep's seminal fluid, but shows a marked tachyphylactic reaction towards PGE.

From the results obtained it is concluded that the prostaglandin preparations used in this investigation (*e. g.* prostaglandin extracted from sheep's vesicular glands under various conditions, from sheep's seminal fluid, and from human seminal fluid) neither can be identical with PGE or PGF, nor can be a combination of only these two factors.

VESIGLANDIN

In semen or extracts from the vesicular glands or the prostate of monkey EULER (1935 a, 1936) demonstrated an acid substance which was similar to prostaglandin. The active principle, vesiglandin, was, however, clearly separated from prostaglandin extracted from sheep's vesicular glands, since it was less stable in acids and alkali, and had a weak action on the atropinized isolated rabbit jejunum compared to that on the rabbit blood pressure.

DARMSTOFF

This factor which has been extracted from intestinal preparations of various animals, is lipid soluble and possesses acidic properties. It has been assumed to be an acetalphosphatidic acid (VOGT 1949, 1955, 1957).

Preliminary experiments were performed in the present investigation in order to compare the biological effects of prostaglandin and Darmstoff (kindly supplied by Dr. W. VOGT). It was, however, found that the Darmstoff preparation contained a factor, which interferred with the bioassay, *e. g.* on the guinea-pig ileum. The occurrence of such a factor in this Darmstoff preparation has been confirmed by VOGT (1958 a) and by him presumed to be a lysophosphatidic acid. Darmstoff could, however, be separated from prostaglandin by its lack of blood pressure lowering effect when injected intravenously in rabbits. This observation agrees with the results by VOGT (1956 a).

IRIN

From rabbit iris AMBACHE (1957 a) extracted a smooth muscle stimulating factor with acidic properties. This substance, irin, which in vivo contracts the atropinized rabbit iris, is assumed to be an organic unsaturated acid with hydroxy groups (AMBACHE 1958). Irin can be separated from prostaglandin biologically, since it does not stimulate the isolated atropinized rat uterus. On paper chromatograms run in methylethylketone/diethylamine/water, irin has a Rf-value of 0.9 (AMBACHE, quoted from VOGT 1958 b), while prostaglandin in the same system has a Rf-value of 0.4—0.5 (ELIASSON 1957). Moreover, irin is thermolabile at pH 6 to 7 (AMBACHE 1957 b).

G-ACID

The well known smooth muscle stimulating property of human serum has been shown by Gabr (1956) to be in part due to an organic acid, G-acid, with the characteristics of a straight-chain unsaturated monocarboxylic acid, probably identical with $\triangle$-3-octadecanoic acid. It is distinguished from prostaglandin by its stong hemolytic effect; moreover its calcium salt is not soluble in water, and it has no action upon the isolated rat colon.

PLAIN MUSCLE STIMULANTS IN THE MENSTRUAL FLUID

If the endometrium from human uterus or menstrual fluid is extracted with acetone, ether, or chloroform the extract contains one or more factors which contract various smooth muscle organs (Pickles 1957, Chambers and Pickles 1958). From the available data it does not seem unlikely that the active principle is an organic acid. It can be clearly differentiated from prostaglandin, since it regularly contracts strips from human nonpregnant uterus. The isolated guinea-pig ileum is also contracted by this factor, but when the extract has been washed out it takes several minutes before the guinea-pig ileum returns to the base line. In contrast, after prostaglandin has been washed out from the bath the test organ reaches its resting level within 20 to 40 seconds.

SLOW REACTING SUBSTANCE A

Several substances isolated from various tissues are known to produce a gradual increase in tonus of the isolated guinea-pig ileum in difference to the rapidly acting histamine and acetylcholine. The various known substances which give rise to this slow developing contraction can be divided chemically into two groups: 1) *polypeptides, e. g.* substance P (Euler and Gaddum 1931, Pernow 1953), bradykinin (Rocha e Silva, Beraldo and Rosenfeld 1949), etc.; and 2) *lipid soluble organic acids.*

SRS-A (Slow Reacting Substance in Anaphylaxis) is generally considered to belong to the latter group. It is liberated in anaphylactic reactions, *e. g.* sensitized lung tissue in contact with the antigen (Kellaway and Trethewie 1940, Brocklehurst 1953). A similar principle is also found to be liberated from asthmatic human lungs perfused with allergen solutions (Brocklehurst 1955). According to Brocklehurst (1955, 1956) it has no action on the rabbit's or cat's blood pressure,

on the guinea-pig or rat uterus, or the rat colon in vitro and is on this account separated from prostaglandin.

UVNÄS and co-workers have investigated the mechanism behind the release of histamine and SRS both in anaphylaxis and as produced by compound 48/80. From their results they have come to the conclusion that most likely similar enzymatic processes are involved in the release of histamine and SRS in anaphylaxis and after addition of 48/80 (HÖGBERG and UVNÄS 1957). They have also shown that 48/80 releases histamine and SRS in parallel with each other from perfused cat paw (CHAKRAVARTY, HÖGBERG and UVNÄS 1959) and also from isolated rat mast cells (UVNÄS and THON 1959).

The SRS liberated from various tissue in anaphylactic reactions and by the action of 48/80 seems to differ from prostaglandin in several respects. The threshold dose of SRS on the guinea-pig ileum is about half that on the rabbit jejunum in vitro (CHAKRAVARTY, personal communication), while the threshold dose for prostaglandin from the non-incubated organ on the rabbit jejunum is much less than on the guinea-pig ileum. In aqueous solutions SRS is rapidly destroyed at neutral pH, while prostaglandin under similar conditions is stable even if boiled for 20 minutes. Moreover, compound 48/80 inhibits the release of prostaglandin in ground vesicular glands of sheep.

The SRS liberated by 48/80 has not been examined on other test organs than the isolated guinea-pig and rabbit intestines. There is, however, little reason to believe that the SRS liberated by 48/80 should be different from the SRS-A described by KELLAWAY and TRETHEWIE (1940) and by BROCKLEHURST (1953, 1955, 1956).

SLOW REACTING SUBSTANCE C

When egg yolk is incubated with cobra venom (FELDBERG, HOLDEN and KELLAWAY 1938) or when lecithin from egg yolk is incubated with lecithinase A (VOGT 1956 b, c) a substance is released that contracts the guinea-pig ileum in vitro. The active principle has been called SRS-C. The chemical properties and the conditions of formation support the opinion that it is a fatty acid derived from lecithin. Biologically it has only been characterized by its action on the isolated guinea-pig ileum, which slowly contracts after a latency period of some minutes. After the extracts have been washed out the guinea-pig ileum takes several minutes to reach the base line. Prostaglandin after a latency period of 3 to 10 seconds gives rise to much more rapid increase in tonus and after washing out the guinea-pig ileum returns to the base line within 40 seconds. SRS-C is also differentiated from prostaglandin by its strong hemolytic effect.

Discussion

In accordance with the results in earlier investigations (EULER 1934, 1935 b, 1936, 1939) it has been found that prostaglandin is present in semen from man and sheep. In addition, a prostaglandin-like factor has been found in goat's semen, but due to lack of material a more detailed chemical and biological characterization of this factor has not been possible. Prostaglandin has not been found in semen of the stallion, bull and boar. In sheep it is secreted exclusively from the vesicular glands and the ampulla and not from the other accessory genital glands. It has been possible to demonstrate that also in man prostaglandin is secreted exclusively from the seminal vesicles. This latter finding is at variance with the results by EULER (1934, 1935 b) and BARNES (1939), who found the active principle to be present also in extracts and expressed fluid from human prostate glands. The difference in results is, however, most likely due to contamination of the prostate extracts with fluid from the vesicular glands, which is difficult to prevent (BARNES 1939).

Prostaglandin has not been found in any of the other organs investigated, including the accessory genital glands of dog, cat, rabbit and guinea-pig.

The conclusion that the prostate glands of man and sheep do not contain or secrete prostaglandin makes the name of the active principle inadequate. It would seem expedient, however, to use the name until the chemical identity of the naturally occurring prostaglandin has been established. "Vesiglandin" cannot be used since this name is already applied to a similar but not identical factor in monkey's semen (EULER 1935 a, 1936).

It appears that naturally occurring biologically active substances in general are present in the tissue in a bound inactive form. Experimental data thus strongly suggest that adrenaline and noradrenaline (HILLARP and NILSSON 1954, ELIASSON, EULER and STJÄRNE 1955, EULER 1958), histamine (GROSSBERG and GARCIA-AROCHA 1954, MCINTIRE 1956), and acetylcholine (BRODKIN and ELLIOTT 1953, WHITTAKER 1958) are bound in the tissue with a ionic (salt) type of linkage. In the present investigation various experiments performed with the sheep's ground vesicular glands demonstrated that also prostaglandin is present in the tissue in an inactive form, but most likely bound by an *ester* linkage. At present

it is not possible to decide if this difference in binding is due to the acid properties of prostaglandin or whether it has a more functional significance.

The storage capacity of the human seminal vesicles is relatively small, and one ejaculation almost completely deplete the organ on its fructose content (MANN 1954). Replenishing the fructose then takes about two days. In the present investigation it was found that, in some cases, the seminal vesicles could replete the prostaglandin store within a 14 to 24 hour interval, while in other cases the synthesis was slower.

It has been found (Table II, p. 17) that the prostaglandin content in samples of semen from the same subject is dependent not only upon the length of the preceding abstinence period, but most likely also on other factors, *e. g.* the functional status of the endocrine system regulating the activity of the accessory genital glands, and the subjective sex drive ("libido"). This latter factor most likely influences the volume of the ejaculate, and may also play a role for the fertility capacity of semen as suggested by KERRUISH (1955). He found that bulls with intense sexual stimulation prior to semen collection not only had markedly improved sexual behaviour but also an increased conception rate. A role of higher nervous centers in the control of the seminal discharge was demonstrated in rabbits by CROSS and GLOVER (1958). This finding might indicate the mechanism over which the subjective sex drive can influence the volume and composition of the semen.

Experiments on various animals have shown that testosterone regulates the functional activity of the accessory genital glands. This is evident from analyses of their content of various chemical constituents like fructose, citric acid, and acid phosphatase (MANN 1954). It was therefore unexpected to find in the present study the concentration of prostaglandin and fructose — which both are exclusively secreted from the seminal vesicles — inversely proportional to each other (Fig. 2, p. 15). The reason for this has not been investigated. However, MANN (1956, p. 375) has suggested that the sex hormone may not play an important role in the fructose formation in man, and if this is true the inverse proportionality between prostaglandin and fructose content in this limited number of experiments becomes less confusing.

HAWKINS and LABRUM (1956, 1959) have suggested that prostaglandin might be a factor of importance for fertility in man. If this be true further studies involving various conditions which influence the total amount of prostaglandin in semen would seem to be of great theoretical and clinical interest.

There appears to be three possible physiological functions of prostaglandin.

1. Prostaglandin may act as a chemical stimulator inducing the emptying of the accessory genital glands (Euler 1936).

2. Prostaglandin may be of importance for the motility of the spermatozoa.

3. Prostaglandin may facilitate the migration of spermatozoa in the female genital tract.

Because prostaglandin is present in semen from man, sheep and possibly also goat, but not in semen from cattle, horse, pig and the commonly used laboratory animals, and because there are no obvious anatomical and physiological differences in the ejaculation process between these species, prostaglandin probably does not play a role for the ejaculation process. On the other hand prostaglandin might play a role in facilitating the emptying stimulus when it has reached a certain concentration (Euler 1936).

The correlation between the prostaglandin content and the duration of spermatozoan activity reported by Hawkins and Labrum (1956) might support the second alternative. However, it does not seem likely that the action of prostaglandin should be of great significance under normal conditions because of the short time interval between ejaculation and the presence of spermatozoa high up in the uterine cavity or fallopian tubes. Asplund (1947 a) and Hawkins and Labrum (1956) could not find any correlation between prostaglandin content and number or degree of motility of the spermatozoa in the ejaculate. Eliasson and Olsson (unpublished) found that addition of purified prostaglandin to 16 semen samples selected at random either decreased or did not change the maintenance of the spermatozoan activity as measured by double blind technic during a 24 hour period. Nor did prostaglandin significantly change the oxygen consumption of washed human spermatozoa suspended in Krebs-Ringer phosphate buffer (Eliasson, unpublished). The conclusion is therefore that prostaglandin is not likely to be of importance for the motility of the spermatozoa.

Relevant to the discussion of the possibility of prostaglandin to facilitate spermigration are some known anatomical and physiological differences of the reproductive organs of various animals.

In animals like the horse, pig, dog, rat, and guinea-pig the spermatozoa are ejaculated directly into the uterine cavity often in a large volume of semen plasma. Spermigration therefore seems to be no problem in these species.

However, in cow, sheep, goat, cat, and rabbit the semen is deposited in the vagina and the spermatozoa thus have to move up through the

cervix, uterine cavity and fallopian tube either actively by their own motility, or passively by way of a propulsive action of the uterine motility.

In cows and rabbits it has been possible to demonstrate a rapid passive propulsion of spermatozoa as well as inert material like radio opaque fluid from the vagina to the fallopian tubes. In these two species the ascent of material can be evoked by a variety of factors like mating, artificial stimulation of the genital organs and infusion of oxytocin (VanDemark and Moeller 1951, VanDemark and Hays 1954, Rowson 1955, Parker 1931, Krehbiel and Carstens 1938). The time interval necessary for the spermatozoa to migrate from the vagina to the oviducts in cow was estimated to be about 5 minutes by VanDemark and Moeller (1951).

A passive migration of spermatozoa was also shown to occur in sheep by Schott and Phillips (1941) and Starke (1949), who demonstrated that spermatozoa could be found in the fallopian tubes 20 minutes after normal service of the ram. There seem to be no observations on a passive transportation of inert material in the genital tract of the ewe.

A passive spermigration most likely also occurs in man. This is indicated by the short time interval between ejaculation and the presence of spermatozoa in the uterine cavity (cf. Belonoschkin 1949, Hartman 1957), and by the occurrence of a passive transportation of carbon granules (Amersbach 1930), and carmine granules (Trapl 1943) from vagina into uterus following coitus with orgasm.

It is well known that in animals both psychic and physic sexual stimulation increases uterine motility, and that this increase most likely is due to liberation of oxytocin (Westman 1926, Hays and VanDemark 1953 b, Fitzpatrick 1957 b). According to Gassner (1953), VanDemark and Hays (1954), and Fitzpatrick (1957 b) oxytocin plays an important role for the passive intrauterine propulsion of spermatozoa in rostal direction at least in cows. In these animals Gassner (1953) has been able to demonstrate that during sexual heat, mating, or artificial insemination the uterine contraction waves are reversed in direction, *i. e.* proceeding from the cervical to the fallopian end of the uterus.

Recording of mammary blood flow, milk-ejection and diuresis have made it possible to demonstrate that during coitus, and particularly at orgasm, oxytocin most likely is released also in women (Pickles 1953, Harris and Pickles 1953, Campbell and Petersen 1953, Friberg 1953). These observations are of interest with regard to the results by Amersbach (1930), Trapl (1943) and Belonoschkin (1949) who found orgasm essential for a passive transportation of spermatozoa from vagina into the uterus.

Asplund (1952) observed that during hysterograms made in the middle of the cycle, the radio opaque medium very quickly passed from the uterus into the abdominal cavity in a remarkably large number of cases.

This finding suggests the occurrence of an antiperistaltic movement also in the human uterus. On the other hand there are no indications that these antiperistaltic movements should be able to facilitate the migration of material from the vagina into the uterus.

It therefore appears that in the cow and rabbit passive propulsion of fluid from the vagina to the fallopian tubes is mediated by nervous and/or humoral mechanisms, mating not being necessary for the phenomenon. In man, however, and probably also in sheep, coitus and deposition of semen in the vagina seem to be essential in order to evoke the passive spermigration. The seminal fluid of man and sheep contains a principle, prostaglandin, which has been shown to influence uterine motility. It therefore seems to be of particular interest to analyze if the changes in uterine motility evoked by prostaglandin are such that they could facilitate a passive spermigration in man.

In a recent investigation on healthy fertile women ELIASSON and POSSE (1959) have studied the effect of prostaglandin on the motility of the non-pregnant uterus. The uterine motility has been recorded with KARLSON's (1944) method, using three separate pressure transducers, which simultaneously recorded the pressure changes in the corpus, isthmus, and cervix uteri.

Prostaglandin has been found to cause a decrease in tonus and motility of isthmus and cervix, *i. e.* the effects obtained with prostaglandin in this respect seem to be identical with those previously observed with human semen (KARLSON 1949). The decrease in tonus of the functional sphincters in the isthmus and in the utero-tubal junction in response to semen or prostaglandin would seem to facilitate the passage of the spermatozoa from vagina into fallopian tubes (WESTMAN 1942, RUBIN 1947, ASPLUND 1952, BORELL and FERNSTRÖM 1953, WESTMAN 1955).

During the menstrual cycle there is a change in the spontaneous motility of the myometrium, the frequency of the contractions showing a peak at the time of ovulation (cf. POSSE 1958). Prostaglandin increases the spontaneous activity in the corpus at the time of ovulation, but only rarely has an effect on the motility of other periods of the menstrual cycle (ELIASSON and POSSE 1959). Whether this is due to changes in the relative sensitivity of the myometrium to prostaglandin or to an impaired absorption from the vagina of the active principle has not been investigated.

As mentioned above a release of oxytocin is likely to occur in women during coitus. The effect of prostaglandin on the uterus under influence of oxytocin, infused in doses of 50 to 200 mU per minute, was therefore also studied by ELIASSON and POSSE (1959). The experiments were performed at the presumptive ovulatory date as determined from the basal temperature curve, and from the motility pattern according to the

investigation by Posse (1958). In these cases prostaglandin caused a marked decrease in the activity of all parts of the uterus. In control experiments without oxytocin on the same women prostaglandin showed the usual effect, *i. e.* increased activity in corpus and a decrease of tonus in the isthmus.

In the present investigation it has been shown that prostaglandin in small doses may increase the activity of isolated strips from human non-pregnant uterus, while slightly larger doses decrease the spontaneous activity (Fig. 12, p. 38). Strips from isthmus have also been found to be more sensitive to prostaglandin than strips from corpus uteri.

It has been suggested by Csapo (1954) that oxytocin does not stimulate the uterus in the strict sense of the word but increases the sensitivity of the myometrium both to the "intrinsic" stimuli responsible for the spontaneous motility, and to external stimuli, *e. g.* electrical stimulation. The observation that a small dose of prostaglandin may increase but a slightly larger dose decrease the activity of the myometrium in vitro and the finding that oxytocin may reverse the prostaglandin response of the intact myometrium (Eliasson and Posse 1959) strongly support Csapo's assumption (1954). The qualitative difference in response to prostaglandin shown by the corpus and the isthmus of the human uterus in vivo, as well as that demonstrated for the corpus with and without stimulation of oxytocin, therefore appears to depend upon functional variations in the responsiveness of the preparation (cf. also Fig. 12 A, p. 38).

From the various results obtained in the experiments with prostaglandin extracted from human semen it can be concluded that this factor shows the same effects on the human uterus in vivo and in vitro as does native semen. It appears most likely that the reactivity pattern of the uterus to prostaglandin observed in vivo, during a simultaneous infusion of small amounts of oxytocin, is similar to that which occurs physiologically, when semen is deposited in the vagina at coitus. This suggestion is supported by the observations by Bickers and Main (1941) and by Karlson (1949). The former authors reported that uterine motility was temporarily abolished following coitus in the human, and Karlson (1949) could demonstrate a cessation of uterine motility in one patient during artificial insemination.

If there is an increase in tonus of the uterus at coitus and orgasm followed by a marked decrease in tonus evoked by prostaglandin 2 to 5 minutes after the ejaculation, this would seem likely to facilitate a passive spermigration from the semen pool around the portio into the uterine cavity.

On the basis of the comparative physiology and reproduction of various animals and man and of the experimental data obtained with prosta-

glandin, the hypothesis is propounded that the physiological function of prostaglandin is to facilitate the passive migration of spermatozoa from the vagina into uterus.

According to this hypothesis one would expect that if prostaglandin for some reason would induce contraction instead of relaxation of the myometrium at coitus, this would make spermigration difficult and perhaps even constitute a factor in infertility.

The role of the motility pattern of the human uterus for spermigration has been brought out by BICKERS (1951). In a small number of examined infertile patients he demonstrated hypertonic, arrhythmic contractions of the myometrium at the time of ovulation. In these patients he also could demonstrate that the spermigration was markedly lessened as compared with the same phenomenon in fertile women with normal uterine motility.

With regard to the similarities in reaction pattern of human uterus in vivo and in vitro following prostaglandin, it would seem justified to bring forward the observations by KURZROK and LIEB (1930) and COCKRILL, MILLER and KURZROK (1935). These authors investigated the effect of human semen on isolated strips of non-pregnant uterus from more than 400 patients. The uterus normal response was relaxation (similar to that illustrated in Fig. 13, p. 39). However, a small number of the preparations responded consistently with increase in tonus to semen. It was found that the hyperactive uteri were obtained from patients with a history of prolonged or complete infertility. BICKERS' (1951) observation, discussed above, would indicate that the hyperactivity of these isolated preparations to semen, also might have been associated with a delayed spermigration in vivo. There is no reason to believe that the increase in tonus in a small number of the uterine preparations observed by KURZROK and co-workers should have been due to a factor other than prostaglandin in the semen samples used.

In the present investigation it has been shown that the sensitivity of various preparations to prostaglandin is dependent not only upon species differences but also on the influence of the ovarian hormones on the myometrium. Isolated strips of human non-pregnant uterus are extremely sensitive to prostaglandin, 0.002 units per ml bath fluid being a usual threshold dose at the time of ovulation. The sensitivity of the isolated rabbit uterus in oestrus is almost of the same order of magnitude, 0.005 units per ml bath fluid being a usual threshold dose. The rat uterus on the other hand is about 40 times less sensitive than the rabbit uterus.

The reactivity pattern of the uterus in vivo and in vitro to different stimuli is influenced by a variety of conditions. Thus it has been shown that the response to adrenaline of the uterus of cat, rabbit, sheep and cow

in vivo as well as in vitro is dependent on the hormonal status. With adrenaline and noradrenaline the oestrogen stimulated uterus responds with decrease in activity and the progesterone stimulated uterus with increase in activity (ROBSON and SHILD 1938, REYNOLDS 1949, FITZPATRICK 1957 a, 1958). The latter author has also shown that depending upon the relative proportion of the actions on the myometrium of the ovarian hormones all forms of transitions between these two extremities can be recorded.

The importance of the hormonal status for the behaviour pattern of the rabbit's uterus to electrical stimulation has been investigated by CSAPO and CORNER (1952) and SCHOFIELD (1954, 1955). The changes in reactions to electrical stimulation with regard to the dominating ovarian hormone, can experimentally be related to alterations in the intracellular cation balance (CORNER and CSAPO 1953, HORWATH 1954). The sympatico-adrenal discharge also plays an important role for the motility and for the behaviour of the uterus following various stimuli as has been shown by SCHOFIELD (1952), HAYS and VANDEMARK (1953 a), VANDEMARK and HAYS (1954), BICKERS (1956), CROSS (1958), and others.

The possible role of the acid-base balance for the uterine reactivity pattern does not seem to have been investigated and there is only meagre information on the role of the initial tonus of the organ for the effect elicited by different stimuli (RUNGE 1928).

It has been shown in the present investigation that the rabbit's uterus gives inconsistent reponse to prostaglandin, and there are, in addition to hormone pretreatment, other factors of importance in the reactivity pattern of uterus following prostaglandin. With regard to the assumption that hyperactivity of the human uterus might be a cause of infertility, the reason for such a reversal in response warrents further studies. The rabbit's uterus might prove suitable for investigations planned to elucidate some of the factors which may influence the reactivity pattern of the myometrium following prostaglandin.

Summary

1. A study has been made of the occurrence, formation and biological actions of prostaglandin. The biological activity is expressed in units of a standard, prepared from sheep's non-incubated vesicular glands.

2. It was found that the prostaglandin present in seminal fluid from man is secreted exclusively from the seminal vesicles. This was shown by analysis of various constituents in the different fractions of split ejaculates. Except in the vesicular glands of sheep and the semen of sheep and goat prostaglandin did not occur in any of the other organs or semen samples investigated.

3. The active principle is present in the tissue in an inactive form, and is secreted into the seminal fluid in its free, active state. Unlike the biologically active amines, which appear to be bound in the tissue by ionic links, prostaglandin most likely is bound by an ester linkage.

4. Analysis of successive samples of human semen have shown that the seminal vesicles, in some cases, are capable of replenishing their content of prostaglandin within a 14 to 24 hour interval. In other cases the synthesis was slower. The reason for this difference is discussed.

5. With appropriate conditions of incubation the amounts of free active prostaglandin in sheep's ground vesicular glands can be increased from 2 to about 80 units per gram tissue.

6. A number of factors influencing the yield of prostaglandin from sheep's ground vesicular glands has been studied, such as temperature, pH, volume of buffer, and various substances. Cobra venom, enzymes with esterase activity, and detergents increase, while compound 48/80 decreases the yield of prostaglandin.

7. Prostaglandin from human semen and from sheep's non-incubated vesicular glands behaved as identical substances in various chemical, chromatographic, and biological tests. Prostaglandin from sheep's semen and incubated vesicular glands were also identical with each other. However, between the two sets, there were minor biological differences. Whether these are due to the active principle or to the occurrence in the latter extracts of an additional factor, chemically related to the prostaglandin, is discussed.

8. Prostaglandin in most cases produces an increase in activity and tonus of various smooth muscle organs. However, this is not the case with isolated human nonpregnant uterus, where it causes a decrease in

activity and tonus, the threshold dose being about 0.002 units prostaglandin per ml bath fluid. The action of native human semen and of prostaglandin extracted from this fluid are of the same character on isolated strips from human nonpregnant uterus.

9. Tests on the isolated rabbit uterus show inconsistent results and an increase as well as a decrease in activity and tonus can be observed after addition of prostaglandin.

10. A change in the spontaneous motility is usually observed on rabbit's uterus in situ, but also here the response to prostaglandin is qualitatively inconsistent. Both increase and decrease in the activity have been observed even in the same test preparation. The possible reason for this variability is discussed.

11. On the basis of its chemical and biological properties prostaglandin can be differentiated from all other known naturally occurring smooth muscle stimulating substances, including the crystalline compounds prostaglandin E and F, and the so-called slow reacting substances.

12. The possible physiological function of prostaglandin in man is discussed and it is suggested, that the action of prostaglandin on the human nonpregnant uterus in vivo facilitates the spermigration in the uterus.

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